New Brunswick
an eppendorf company

Guide to Operations

CelliGen® BLU
Single-Use Bioreactor

MANUAL No: M1363-0050
Revision C
January 18, 2011

New Brunswick Scientific **PO Box 4005 44 Talmadge Rd. Edison, 08818-4005 USA**
1.800.631.5417 1.732.287.1200 bioinfo@nbsc.com www.nbsc.com

CelliGen®, Excella®, Innova® and BioCommand® are registered trademarks of and are owned by New Brunswick Scientific Co., Inc., USA.

CLAVE® NeedleFree is a registered trademark of ICU Medical, Inc. in San Clemente, California, USA.

Luer-Lock™ is a trademark of Becton, Dickinson and Company in Franklin Lakes, New Jersey, USA.

Tri-Clamp® is a registered trademark of Ladish Co. Corporation, Alfa Laval, Inc., Richmond, Virginia, USA.

Windows® is a registered trademark of Microsoft Corporation in the United States and other countries.

New Brunswick Scientific has attempted to identify the ownership of all trademarks from public records. Any omissions or errors are unintentional.

CONTACT US:

New Brunswick Scientific maintains regional sales and service offices throughout the world to best serve you.

To locate the office nearest you, see the “Contact Us” section of our website at www.nbsc.com, or contact us at our world headquarters:

New Brunswick Scientific
44 Talmadge Road
Post Office Box 4005
Edison, New Jersey 08818-4005 USA

Tel. +1.732.287.1200
Toll-free in North America: +1.800.631.5417
Fax: +1.723.287.4222
Email: bioinfo@nbsc.com
Website: www.nbsc.com

WARNING!
High voltage.
Always make sure this equipment is properly grounded.

WARNING!
This product is not designed to contain gases within the range of their lower explosion limit (LEL) and their upper explosion limit (UEL).
If your process requires or produces gases, be sure to verify their LEL and UEL concentration range (available online).

CAUTION!
This equipment *must* be operated as described in this manual. If operational guidelines are not followed, equipment damage and personal injury *can* occur. Please read the entire User's Guide before attempting to use this unit.

Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.

New Brunswick Scientific Co., Inc. (NBS) is not responsible for any damage to this equipment that may result from the use of an accessory not manufactured by NBS.

Copyright Notice

New Brunswick Scientific
Box 4005 44 Talmadge Road
Edison, New Jersey 08818-4005

© Copyright 2010 New Brunswick Scientific

All Rights Reserved.
Reproduction, adaptation, or translation without prior written permission from New Brunswick Scientific is prohibited.

Disclaimer Notice

New Brunswick Scientific reserves the right to change information in this document without notice. Updates to information in this document reflect our commitment to continuing product development and improvement.

Manual Conventions

NOTE: **Notes contain essential information that deserves special attention.**

CAUTION! ***Caution* messages appear before procedures which, if caution is not observed, could result in damage to the equipment.**

WARNING! ***Warning* messages alert you to specific procedures or practices which, if not followed correctly, could result in serious personal injury.**

WARNING! **This particular *Warning* message represents a potential electrical hazard.**

WARNING! **This particular *Warning* message, whether found in the manual or on the unit, means HOT SURFACE–and therefore represents a potential danger to touch.**

CRUSH WARNING! ***Crush Warning* messages alert you to specific procedures or practices regarding heavy objects which, if not followed correctly, could result in serious personal injury .**

WARRANTY

New Brunswick Scientific's equipment is protected by a comprehensive warranty. The warranty covers faulty components and assembly, and our obligation under this warranty is limited to repairing or replacing the instrument or part thereof, which shall prove to be defective after our examination.

The NBS warranty does not cover loss of time or materials, such as the loss of biological or biochemical by-products caused by any work interruption resulting from equipment failure; it does not extend to equipment that has been subject to misuse, neglect, accident or improper installation or application; nor does it cover any machine that has been repaired or altered by anyone other than an authorized NBS factory-trained service representative, without prior approval from your local NBS sales office.

Expendable items such as bearings and seals, lamps, probes, sensors including incubator sensors, glass, filters, etc., are not covered.

The warranty begins on the date the equipment ships and extends through the period indicated in the chart below:

Instrument		Parts Warranty	Labor Warranty
Shakers	Innova®	3 years	2 years
	I Series	2 years	2 years
	Excella® & C-76	2 years	2 years
	Accessories[1]	1 year	1 year
CO_2 Incubators	Incubators	2 years	2 years
	Accessories[2]	1 year	1 year
Freezers	ULT Freezers	5 years; vacuum insulation panels: 12 years	2 years
	Accessories[3]	1 year	1 year
Fermentors, Bioreactors & all other NBS equipment		1 year	1 year

1 Chart recorders, photosynthetic light banks, etc.
2 Stacking stand, casters, shelves, etc.
3 Chart recorders, CO_2/LN_2 back-up systems, etc.

Warranty Registration

To register your warranty with NBS, complete the online form at www.nbsc.com under the **How Can We Help?** tab, or use the warranty card enclosed with your equipment.

Extended Warranty Option

A variety of service plans are offered to help minimize downtime from unexpected malfunctions in equipment operation. Speak to your NBS sales representative for more information.

BIOREACTOR

INFORMATION SHEET

On this page, record the information for your bioreactor and retain this for future reference.

MODEL NUMBER: _____
VOLTAGE: _____
SERIAL NUMBER: _____

The above information can be found on the electrical specification plate.

Purchased with the following installed options:

_____
_____
_____
_____
_____

TABLE OF CONTENTS

1 WARNINGS & CAUTIONS 1

1.1 WARNINGS 1
1.2 CAUTIONS 1

2 INSPECTION & UNPACKING OF EQUIPMENT 3

2.1 INSPECTION OF BOX(ES) 3
2.2 PACKING LIST VERIFICATION 3
2.3 BASIC COMPONENTS 3

3 INTRODUCTION & OVERVIEW 4

3.1 SYSTEM 4
3.2 VESSELS 4
3.3 AGITATION SYSTEM 4
3.4 TEMPERATURE CONTROL 5
3.5 AERATION 5
3.6 PUMPS 5
3.7 pH CONTROL 6
3.8 DO CONTROL 6
3.9 EXHAUST SYSTEM 6
3.10 SAMPLING SYSTEM 7
3.11 OVERLAY SYSTEM 7
3.12 SCALE (OPTIONAL) 7
3.13 BAG HANGER 7
3.14 RECOMMENDED ACCESSORIES & SUPPLIES 7
3.15 SUPERVISORY SOFTWARE 8

4 INSTALLATION 9

4.1 PHYSICAL LOCATION 9
4.2 ENVIRONMENT 9
4.3 INSTALLING THE CONTROL CABINET 9
4.4 INSTALLING THE TOUCHSCREEN 10
4.5 UTILITIES 11
4.5.1 Electrical Requirements 13
4.5.2 Gas Connections 13
4.6 VESSEL ASSEMBLY 14
4.6.1 Unpacking the Vessel 14
4.6.2 Headplate Penetrations 16
4.6.3 Heat Blanket & Exhaust Tube Heat Blanket 18
4.6.4 Internal Components 19
4.6.5 Inspect the pH Probe 19
4.6.6 Install the pH Probe 19
4.6.7 Inspecting the DO Probe 20
4.6.8 DO Probe Preparation 20

4.6.9 Install DO Probe 21
4.6.10 Install Temperature (RTD) Probe 21
4.6.11 Install Vessel 21
4.6.12 Install Motor Assembly 21
4.7 MAIN POWER SWITCH 22
4.8 OPTIONAL BIOCOMMAND SOFTWARE 22
4.9 INPUTS/OUTPUTS FOR ANCILLARY DEVICES 22
4.10 INSTALL THE BAG HANGER 24

5 SPECIFICATIONS 25

5.1 VALIDATION DOCUMENTATION 26
5.2 CERTIFICATIONS 26

6 OPERATING CONTROLS 28

6.1 TOUCHSCREEN 28
6.2 DISPLAY SCREENS 28
6.2.1 Touchscreen Calibration 28
6.2.2 Start-Up Screen 29
6.2.3 Summary Screen 29
6.2.4 Synoptic Screen 31
6.2.5 Gauge Screens 32
6.2.6 Adding Loops 33
6.2.7 Deleting Loops 35
6.2.8 Selecting Loop Control Modes 36
6.2.9 Calibration Screen 37
6.2.10 Cascade Screen 38
6.2.11 Trend Screen 38
6.2.12 Pumps Screen 39
6.2.13 Alarms Screen 40
6.2.14 Setup Screen 40
6.3 RS232/422 COMPUTER INTERFACE 43

7 PREPARATION & CALIBRATIONS 45

7.1 PH CALIBRATION 45
7.2 DO PROBE POLARIZATION 47
7.3 DO PROBE CALIBRATION: SETTING ZERO 47
7.4 DO PROBE CALIBRATION: SETTING SPAN 48
7.5 SCALE/VOLUME CALIBRATION 49

8 CONNECTING THE VESSEL ASSEMBLY 51

8.1 CONNECT THE VESSEL ASSEMBLY 51
8.2 LOAD PUMP TUBING 51
8.3 INSTALL LIQUID ADDITION SYSTEMS 52
8.3.1 Addition Tubing Size 53
8.4 CONNECT GASES 53

9 GETTING STARTED 55

9.1 CONTROL MODES 55
9.2 SETTING P & I VALUES 55
9.3 LOOP SETPOINTS 55
9.3.1 Entering Setpoints 55
9.3.2 Modifying Setpoints 57
9.4 CASCADE SYSTEM 57
9.5 PUMP ASSIGNMENT 57
9.6 PUMP CALIBRATION 58
9.7 USING OPTIONAL SCALES TO PROGRAM PUMPS 58
9.7.1 Setting a Feed Pump to Add Liquid 58
9.7.2 Setting a Pump to Harvest Vessel Contents 59

10 CASCADE CONTROL 60

10.1 CREATING A CASCADE 61
10.2 CONTROLLING DO BY CASCADE 63

11 GAS MIXING FOR PH & DO CONTROL 65

11.1 GAS CONTROL WITH 0 TMFC 65
11.2 GAS CONTROL WITH 1 TMFC 69
11.3 GAS CONTROL WITH 3 TMFCS 73
11.4 GAS CONTROL WITH 4 TMFCS 75
11.5 GAS OVERLAY MIXING 79

12 PLOTTING TRENDS 81

12.1 CREATING A TREND GRAPH 81
12.1.1 Using the Export Button 84
12.1.2 Using the Zoom Button 84
12.1.3 Using the Read Line 85

13 ABOUT PUMPS 86

13.1 PUMP SETPOINT 86
13.2 PUMP CONTROL MODE 87
13.3 PUMP FLOW RATE & CALIBRATION METHODS 88
13.4 PUMP PERIOD 89
13.5 INSTALLING AN EXTERNAL VARIABLE SPEED PUMP 89

14 ABOUT ALARMS 92

14.1 ABS AND DEV ALARMS 92
14.2 SETTING ALARMS 92
14.3 ACKNOWLEDGING AN ALARM 94
14.4 ALARMS HISTORY 95

15 USING THE SETUP SCREEN 97

15.1 CONTROLLER SETUP 97
15.2 RECIPE MANAGER 98
15.3 SYSTEM SETTINGS 99
15.3.1 Resetting Date/Time 100

15.3.2 Updating Software 100
15.4 HARDWARE SETUP 101
15.5 SECURITY SETTINGS 101

16 PERFORMING A CELL CULTURE RUN 104

16.1 PREPARING FOR START-UP 104
16.2 INOCULATION 105
16.3 START BIOCOMMAND (IF PRESENT) 106
16.4 SAMPLING THE VESSEL 106
16.5 SHUTDOWN 107

17 CLEANING 108

18 MAINTENANCE 109

18.1 PROBE MAINTENANCE AND STORAGE 109
18.2 PERIODIC INSPECTION 109
18.2.1 Motor Assembly Replacement 110
18.3 FUSE REPLACEMENT 110

19 SERVICE 111

19.1 TROUBLESHOOTING 111
19.2 PRODUCT RETURN 112

20 DRAWINGS 113

20.1 LIST OF DRAWINGS 129
20.2 LIST OF TABLES 131

21 APPENDIX A: STACKABLE PUMPS 132

22 APPENDIX B: SOME GENERAL CONCEPTS 134

22.1 WHAT IS A CONTROLLER? 134
22.2 WHAT IS A CONTROL LOOP? 134
22.3 WHAT IS PROBE CALIBRATION? 135
22.4 WHAT ARE P-I-D CONSTANTS? 135
22.5 WHAT IS P-I-D TUNING? 136
22.6 WHAT DO THE CONSTANTS MEAN? 137

23 APPENDIX C: CORROSION RESISTANCE 138

24 APPENDIX D: PROTOCOL FOR GROWING CHO CELLS 139

24.1 ABSTRACT 139
24.2 INTRODUCTION 139
24.3 MATERIALS AND METHODS 140
24.3.1 Single-Use Vessel 140
24.3.2 Controller 140
24.3.3 Inoculum Preparation 140
24.3.4 Bioreactor Set-Up & Inoculation 141
24.3.5 Operational Parameters 141

24.4 RESULTS AND DISCUSSION 143
25 APPENDIX E: PRODUCT RETURNS 145
25.1 RETURN PROCEDURE 145
25.2 RETURN AUTHORIZATION AND DECONTAMINATION CERTIFICATE 145
26 INDEX 147

1 WARNINGS & CAUTIONS

The following section is a recap of all WARNING and CAUTION messages contained in this manual. This information is essential to the safe operation of your CelliGen BLU. Please take a moment to acquaint yourself with the content of each message.

Page numbers are provided so you can review the message and its application within its overall context.

1.1 WARNINGS

WARNING	Page
High voltage. Always make sure this equipment is properly grounded.	iv, 13
This product is not designed to contain gases within the range of their LEL & UEL.	iv
NEVER block the exhaust to pressurize the vessel.	6
Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.	11, 14
NEVER OVER-PRESSURIZE A CULTURE VESSEL!	54
Always turn your CelliGen BLU off and disconnect the power cord before performing maintenance.	109, 111
NO ONE BUT A PROFESSIONAL SERVICE PERSON should touch electric or electronic parts or assemblies in the electrical cabinet.	110

1.2 CAUTIONS

CAUTION	Page
This equipment *must* be operated as described in this manual. If operational guidelines are not followed, equipment damage and personal injury can occur. Please read the entire User's Guide before attempting to use this unit. **Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.** NBS is not responsible for any damage to this equipment that may result from the use of an accessory not manufactured by NBS.	iv
Do not expose the pH sensor within the vessel to direct light or to any liquid with less than 100 mM ionic strength…	6
Do not open your presterilized vessel package until you are ready to use it.	9
Before making electrical connections, verify that the supply voltage matches the voltage and the power requirements marked on the electrical specification plate (located on the rear panel of the cabinet) and the control schematics supplied with the unit.	10, 13

...continued...

CAUTION	Page
Do not open any port in the vessel headplate until ready for use…	14
Never turn on the heat blanket or exhaust tube heat blanket without first plugging in the RTD and inserting it in the thermowell inside the vessel…	18
Before turning on the main power switch, make sure that the power cord is properly connected…	22
Do not allow the combined weight of the bags and their contents to exceed 15 kg (33 lb).	24
Never attempt to operate the system if the DO probe is not installed…	47
Proper pH control is critically dependent on tubing size…	53
When selecting an external pump to operate with your system, please consult with your local sales representative to be sure the model you choose is compatible with your CelliGen BLU.	89
Be sure to set the dip switches correctly when using either 4-20mA or 0-5V inputs/outputs.	91
Acknowledging alarms is NOT a replacement for correcting the condition that triggered the alarm. Diagnose the cause of the alarm condition and rectify the situation to ensure proper operation of your CelliGen BLU.	95
Agitation can only be increased 25 RPM at a time; any greater increase will cause the magnetic drive to uncouple.	104, 105
Never let a probe rest on its tip.	109

2 INSPECTION & UNPACKING OF EQUIPMENT

2.1 Inspection of Box(es)

When you have received your order from New Brunswick Scientific, carefully inspect all parts of the shipment for damage that may have occurred during shipping. Report any damage immediately to the carrier and to your local NBS Sales Order Department.

2.2 Packing List Verification

Verify against your NBS packing list that you have received the correct materials. Report any missing parts to your local NBS Sales Order Department.

2.3 Basic Components

You should have at least the following components, which will be described in greater detail later in this manual:

- Vessel (complete assembly with tubing & connectors)
- Control Cabinet
- Touchscreen
- Heater Blanket
- Probes (RTD, pH & DO)
- Motor
- Exhaust Heater with support rod

NOTE:

The assembled Control Cabinet/Touchscreen assembly is called a Control Station. For purposes of clarity in this manual, however, the control cabinet (which houses the controller) and the touchscreen will be referred to separately by their component names.

3 INTRODUCTION & OVERVIEW

3.1 System

CelliGen® BLU is a versatile benchtop bioreactor that provides a fully equipped system in one compact package, with its Reactor Process Control (RPC) software in cell culture mode and a 15-inch color touchscreen monitor as the user interface. It can be employed for batch, fed batch, or continuous culture with control of 4-gas mixing, pH, dissolved oxygen (DO), agitation, temperature, pump feed, and additional analog/digital inputs and outputs.

3.2 Vessels

The CelliGen BLU cell culture vessel is a single-use stirred tank bioreactor designed for the growth of mammalian cells.

Each 5.0- or 14.0-liter preassembled vessel consists of a vessel body, headplate, pitched blade impeller with shaft, and a retaining ring which supports all the internal tubing. Ports are provided in the headplate for the following purposes: addition (3 ports); a thermowell for a resistance temperature detector (RTD); a sparger; a harvest tube; a sampler; an exhaust tube; and dissolved oxygen (DO) and pH sensors. There is also a port for optional overlay use. All ports and tubing come pre-installed in the vessel. The magnetic drive coupling is also located on the headplate *(see Figures 4a & 4b for more details)*.

Each vessel comes pre-sterilized with all tubing and filters already attached. The tubing used for addition, sampling and harvesting lines can be safely welded in a tube welder.

3.3 Agitation System

A non-disposable, removable agitation motor located on top of the bearing housing on the headplate is connected to the agitation shaft with a magnetic coupling. The magnetic coupling on the headplate has a notch to ensure correct and easy orientation as the motor is installed.

The motor can provide a speed range from 25 to 200 RPM. The process control software ensures agitation speed accuracy throughout the speed range.

Default P & I (proportional & integral) values are preset at the factory. **We strongly recommend that you maintain the factory-set parameters.**

3.4 Temperature Control

The culture temperature setpoint is controlled within the range of 5°C above ambient temperature to 40°C by the process control software. The media temperature is sensed by a Resistance Temperature Detector (RTD) submerged in the thermowell. Temperature will be maintained or adjusted by ambient cooling and heating through the use of a silicone heat blanket.

Default P & I (proportional & integral) values are preset at the factory. **We strongly recommend that you maintain the factory-set parameters.**

3.5 Aeration

Aeration is controlled by inputting values through the touchscreen on the control cabinet. Gas can be introduced by means of a porous sparger or overlay port; the overlay system is optional.

Up to four gases, including air, nitrogen, carbon dioxide and oxygen, can be introduced into the media or headspace. The flow rate is controlled automatically by one, three or four thermal mass flow controller(s); automatic gas mixing can be accomplished by a solenoid bank when only one thermal mass flow controller (TMFC) is present. The TMFC is regulated automatically according to values set via the control cabinet touchscreen. If you wish to use a rotameter, there is an option for no TMFC.

The percentage of gas blended and injected into the vessel can be controlled manually by the user or automatically through the controller. CO_2 can be added to lower pH, and nitrogen can be added to lower DO; for further information on cascading, see Section 10.

Default P & I (proportional & integral) values are preset at the factory. **We strongly recommend that you maintain the factory-set parameters.**

3.6 Pumps

Three Watson Marlow pumps are provided as standard on the right front of the control cabinet. These pumps are very easy to load (*see Section 8.2*). They are all fixed speed peristaltic pumps; Pump 1 is set to 14 RPM and Pumps 2 & 3 are set to 109 RPM.

For more information on selecting setpoints, calibrating flow rates and selecting pulse periods, see Section 13; for information about optional stacked pumps, see Section 0 (Appendix A).

3.7 pH Control

pH is controlled in the range of 6.00-8.00. The pH is sensed by a non-invasive optical pH sensor. Control is maintained by a P & I (proportional & integral) controller which operates peristaltic pumps, assigned to add base or to use of gas(es) for this purpose. The user can also select a deadband value to control pH within the user-assigned range: no base (or gas) will be added when the pH value falls within the deadband tolerance above or below the setpoint.

CAUTION!

Do not expose the pH sensor within the vessel to direct light or to any liquid with less than 100mM ionic strength, as these may adversely affect the performance of the sensor.

Cell culture pH control is typically done by the addition of base by means of a peristaltic pump or by sparging in CO_2 to lower the pH.

Default P & I (proportional & integral) values are preset at the factory. **We strongly recommend that you maintain the factory-set parameters.**

3.8 DO Control

DO is controlled and measured in the range of 0-200% by means of a non-invasive, non-disposable polarographic DO probe. It is sensed by the DO electrode and control is maintained by the P & I controller by adjusting the gas mixture that is injected into the vessel.

Default P & I (proportional & integral) values are preset at the factory. **We strongly recommend that you maintain the factory-set parameters.**

3.9 Exhaust System

The exhaust gases pass into the exhaust tube where the gases are heated up by the exhaust heat blanket; any moisture present there is raised above the dewpoint and can easily pass through the filter. The remaining air passes through the 0.2 μm exhaust filter.

WARNING!

NEVER block the exhaust to pressurize the vessel (*see Section 4.6*).

Pressing the **Exhaust Heater** button at the top right of the control screens (*see Figure 8 for example*) will turn the exhaust heater on or off. **It should always be on when gas is flowing into the vessel.**

3.10 Sampling System

This system consists of a CLAVE® NeedleFree connector attached to a sampling tube that extends to the lower portion of the vessel. Both the CLAVE NeedleFree connector and the syringe use Luer-Lok™ connections. Samples can be taken by aseptically attaching a needle-free syringe to the connector and drawing a sample (*see Section 16.4 for details*).

3.11 Overlay System

The overlay system allows gas to be inserted directly into the headspace of the vessel through a port located on the headplate. Overlays can be selected as manual flow with a rotameter or as automatic flow via a thermal mass flow controller (TMFC). Like the sparge system, the overlay gas mixture can be composed of up to four gases and its composition can be controlled automatically by solenoid valves. *See Section 11.5 for details.*

3.12 Scale (optional)

An optional load cell scale can be added to your system, to display the weight (volume) of your culture vessel or feed vessel. Cascades and feeds can be set up to pump in or pump out liquid automatically based on the scale's process loop information.

3.13 Bag Hanger

A bag hanger is provided so that disposable addition, harvest and/or sample bags can easily be suspended for use in your process. The hanger has three hooks which allow you to hang up to three bags at the right side of the controller (see Section 0 for installation details).

3.14 Recommended Accessories & Supplies

Before you begin to assemble your CelliGen BLU, it would be prudent to verify that you have all of the following accessories and supplies readily at hand:

- Rubber gloves
- C-FLEX® tubing
- Plastic tubing connectors
- A tie gun
- Needle-free syringes
- Media
- Antifoam agent
- Addition bottles

A user's kit is available from NBS with many of the commonly required items (including a selection of tubing, clamps, filters, connectors and addition vessels). Speak to your NBS sales representative for more information.

3.15 Supervisory Software

In addition to the built-in Reactor Process Control (RPC) software that you interface with through the touchscreen, your CelliGen BLU system can be remotely controlled from a PC via NBS *BioCommand*® optional Modbus supervisory software (*see Sections 4.8 and 16.3*) . Consult your NBS representative for details.

4 INSTALLATION

4.1 Physical Location

CAUTION!

Do not open your pre-sterilized vessel package until you are ready to use it.

The surface on which you place the CelliGen BLU bioreactor should be smooth, level and sturdy. Ensure that the surface can bear the weight of the bioreactor plus vessel contents and any applicable ancilliary equipment.

Also ensure that there is enough space around the back and the front of the CelliGen BLU for proper operation and access. Allow at least 4 inches of clearance behind the unit for heat dissipation. See Section 5, *Specifications*, for weights and dimensions.

4.2 Environment

The CelliGen BLU bioreactor operates properly under the following conditions:

- Ambient temperature range 10°C to 30°C
- Relative humidity up to 80% non-condensing

4.3 Installing the Control Cabinet

Position the CelliGen BLU control cabinet on a firm, level surface in an area where utilities are readily available.

Level the horizontal surface of the base with four leveling glides if necessary.

Connect the power cord to the rear of the control cabinet. At a later time, once the unit is completely assembled and all connections have been made, you will plug the power cord into a suitable electrical outlet.

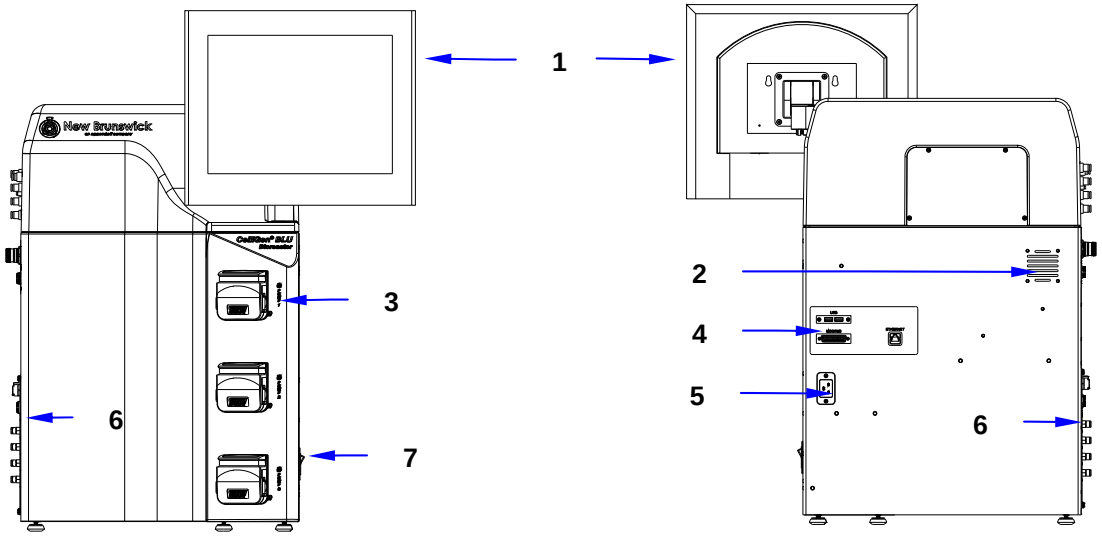
4.4 Installing the Touchscreen

With reference to Figures 1a (for location) & 1b (for installation), align the monitor with the mounting rack on the cabinet, and use the four screws provided with the monitor to securely fasten it to the rack. The mounting rack swivels for easy access.

CAUTION!

Before making electrical connections, verify that the supply voltage matches the voltage and the power requirements marked on the electrical specification plate (located on the rear panel of the cabinet) and the control schematics supplied with the unit.

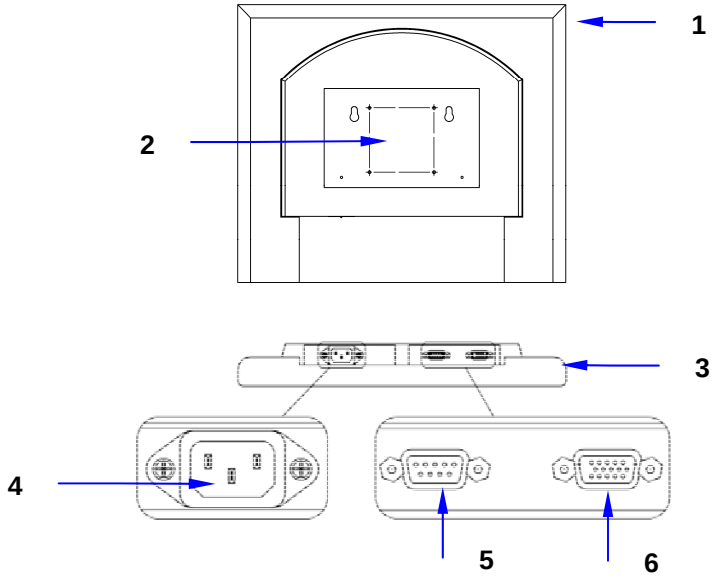
Figure 1a: Front & Rear Views of Control Cabinet



1	Touchscreen	5	Power cord connection
2	Heat vent	6	Service connections (see Figure 2, Section 4.5)
3	Pumps		
4	See Figure 6, Section 4.9	7	Power switch (ON/OFF)

With reference to Figure 1b on the following page, connect the cabinet's power cord plug, com port connector and VGA monitor connector to the bottom of the touchscreen monitor:

Figure 1b: Touchscreen-to-Control Cabinet Connections



1	Touchscreen (rear view)	3	Touchscreen (bottom view)
2	Attach the monitor to the control cabinet mounting rack with the 4 screws provided, using these 4 holes.	4	Power cord plug
		5	COM port connector
		6	VGA monitor connector

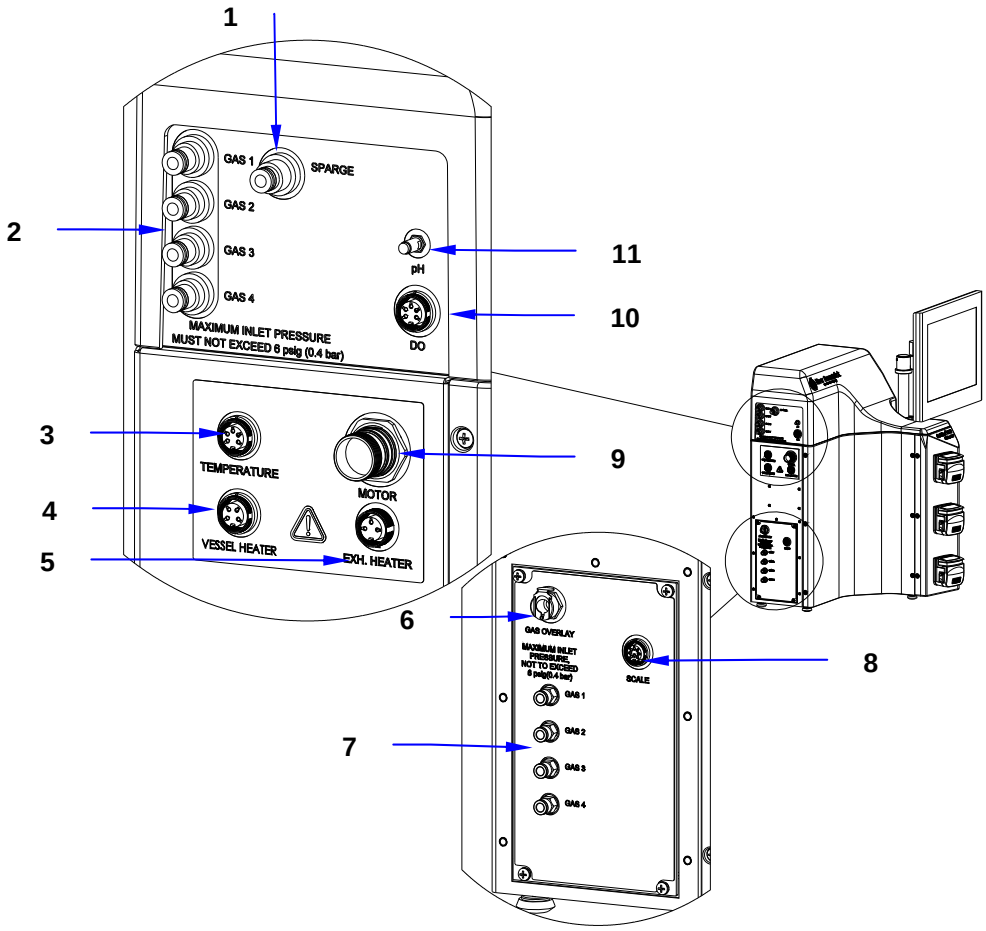
4.5 Utilities

WARNING!

Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.

The control cabinet assembly must be properly connected to gases and electrical power. All gas connections are located on the lefthand side of the cabinet (*see Figure 2 on the following page*). The power connection is located on the back of the cabinet (*see Figure 1a*).

Figure 2: Control Cabinet Service Connections



1	Sparge outlet connection	7	Inlets for overlay gases
2	GAS1–GAS4 inlets for gas connections	8	Scale connector (optional)
3	RTD temperature probe connection	9	Motor cable connection
4	Vessel heat blanket connection	10	DO probe cable connection
5	Exhaust heater connection	11	pH probe cable connection
6	Outlet to Overlay port on headplate		

Using standard plant practices and respecting all applicable codes, connect services to the appropriate connections, as recapped in Table 1 and explained in greater detail in Sections 4.5.1 - 4.5.2.

Table 1: Service Connections

Service/Utility	*Requirement*	*Connection*
Electrical	100-120 VAC, 50/60 Hz., Single Phase, 15 Amp (fluctuations not to exceed ±10%)	100-120 VAC 1ph field wired to 15 Amp disconnect in panel
	200-240 VAC, 50/60 Hz., Single Phase, 15 Amp (fluctuations not to exceed ±10%)	200-240 VAC 1ph field wired to 15 Amp disconnect in panel
Process Air	5-6 PSIG	Push on
Oxygen	5-6 PSIG	Push on
Nitrogen	5-6 PSIG	Push on
Carbon Dioxide	5-6 PSIG	Push on

4.5.1 Electrical Requirements

100-120 Volts	50/60 Hertz	15 Amp
200-240 Volts	50/60 Hertz	15 Amp

NOTE:

The electrical requirements vary depending on the part number that has been ordered. Model, Part Number and Electrical Power Requirements for each bioreactor appear on a metal label affixed to the rear of the unit just above the connection for the power cord.

CAUTION!

Before making electrical connections, verify that the supply voltage matches the voltage and the power requirements marked on the electrical specification plate (located on the rear panel of the cabinet) and the control schematics supplied with the unit.

WARNING!

High voltage.

Always make sure this equipment is properly grounded.

4.5.2 Gas Connections

Gas inlets for both the sparger and the overlay are located on the left side of the control cabinet *(see Figure 2)*.

There are push-in connectors for air, nitrogen, oxygen and carbon dioxide. These connectors accept flexible tubing, which is supplied with the bioreactor. Other soft, flexible-walled, chemically inert tubing (such as Marprene, Pharmed, etc.) may be used as well.

WARNING!

- **Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.**
- **All gases supplied should be medical grade.**
- **No gas pressure should rise above 6 PSIG (*see also page iii*).**
- **Never leave a gas inlet open; if no tubing will be connected, keep the inlet plugged.**

All gases should be regulated using a two-stage regulator. The scale of the regulator gauge for gases going into the bioreactor should be such that one can regulate pressure between 0-10 PSIG maximum.

4.6 Vessel Assembly

CAUTION!

- **Do not open any port in the vessel headplate until ready for use. Prolonged exposure to light will adversely affect the pH sensor.**
- **Never place the vessel in direct sunlight or in a brightly lit location.**
- **Always keep the vessel wrapped with the heat blanket to avoid premature decay of the pH sensor.**

4.6.1 Unpacking the Vessel

Because the CelliGen BLU vessel is shipped sterile, it is important to carefully follow the directions below:

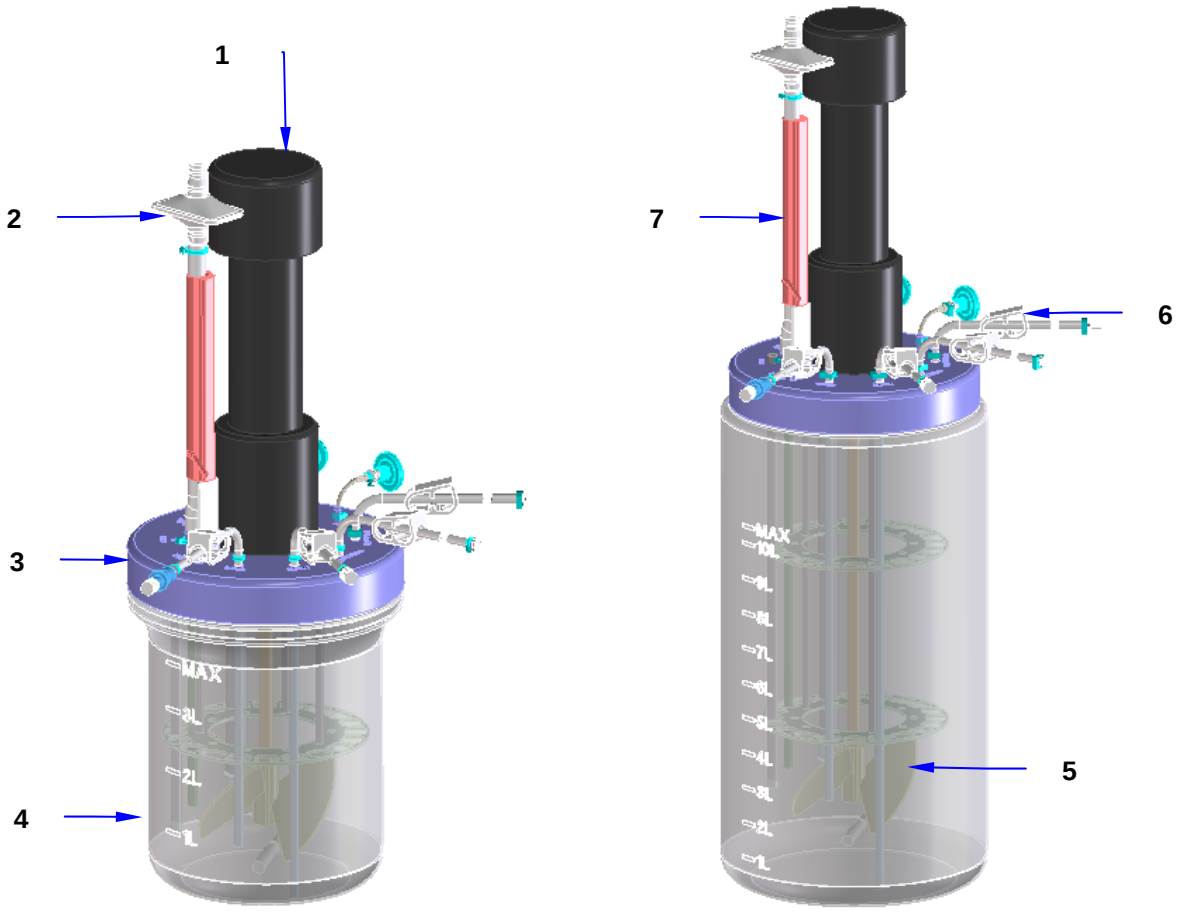
1. Inspect the outer box for damage.
2. Check the label on the box to make sure that the product has not exceeded its expiration period.
3. Open the lid of the box, then remove the cardboard insert by pulling directly up on the cut-out handles.
4. Remove the vessel in its protective opaque outer wrapper. **Do not open the wrapper yet.** Inspect the wrapper for damage.

NOTE:

The outer wrapper is not only a secondary sterile boundary, it is also opaque to protect the pH sensor from light. Do not proceed to Step 5 until you are ready to use the vessel.

5. Carefully tear open the bag at the top. **Do not use sharp blade of any sort.**
6. Carefully slide the vessel out of the protective wrapper, keeping the dome in place to hold all the tubing.
7. Place the vessel next to your control station and install the heater blanket (*see Section 4.6.3*) to protect the sensor from ambient light.
8. Remove the plastic dome and remove all cable ties from the tubing attached to the vessel.

Figure 3: Vessel Assembly

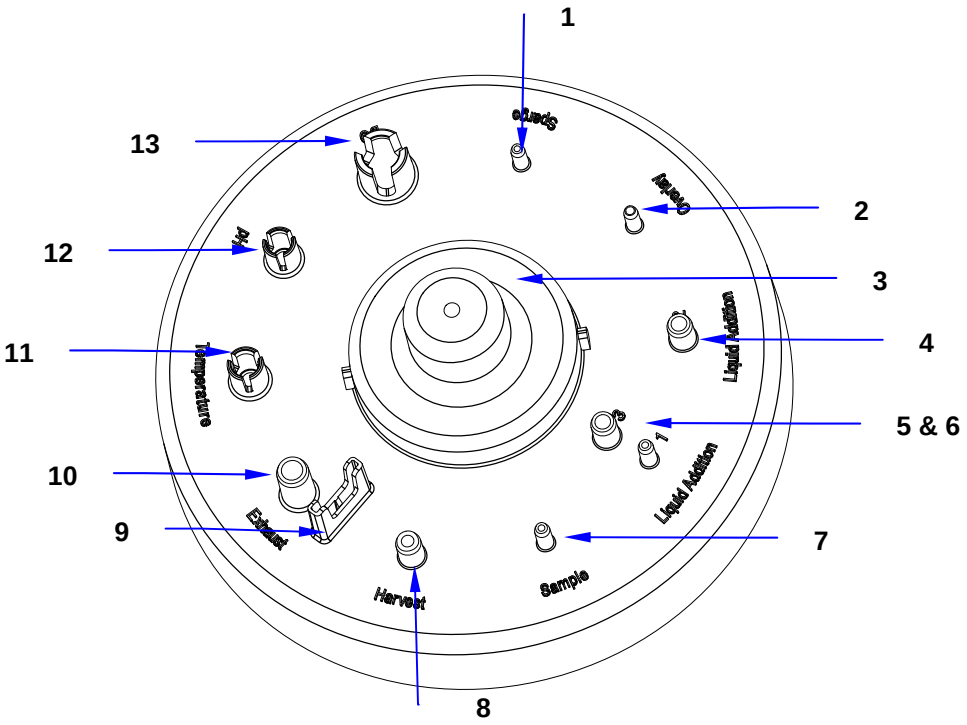


1	Removable/reusable motor	5	Internal components (see Section 4.6.4)
2	Exhaust filter	6	Addition tubes, sample tubes, sparge, overlay (use is optional)
3	Headplate (see Section 4.6.2)		
4	Vessel	7	Heat-blanketed exhaust tube (see 4.6.3)

4.6.2 Headplate Penetrations

Figures 4a & 4b show the port penetrations on the vessel headplate. The ports are the same on both 5L and 14L vessels; they are also labeled on the headplate itself.

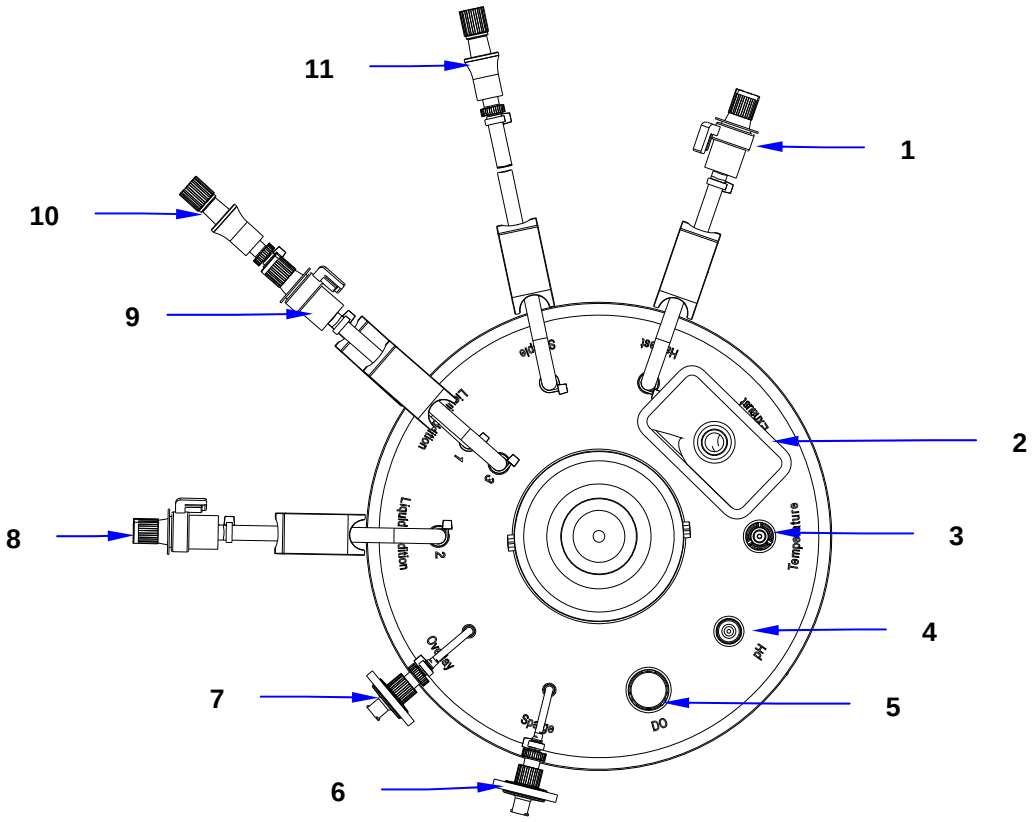
Figure 4a: Headplate Arrangement (shown without tubes & connectors)



1	Sparge port	8	Harvest port
2	Overlay port (use is optional)	9	Exhaust heater support
3	Motor coupling*	10	Exhaust port
4	Addition port 2	11	Temperature port for RTD & thermowell
5	Addition port 3		
6	Addition port 1	12	pH port
7	Sample port	13	DO port

*Note notches for proper alignment

Figure 4b: Headplate Arrangement
(shown with tubes & connectors)



1	¼-inch CPC quick-connect for harvest	7	0.2 μm overlay filter (use is optional)
2	0.2 μm exhaust filter	8	¼-inch CPC quick-connect for Addition 2
3	Thermowell port for RTD	9	¼-inch CPC quick-connect for Addition 3
4	Port for optical pH probe	10	⅛-inch Luer-Lok™ connect for Addition 1
5	Port for polargraphic DO probe	11	Clave® NeedleFree connector for sampling
6	0.2 μm sparge filter		

Table 2 on the following page summarizes the type, length and size (inner and outer diameter) of all the vessel headplate tubing and their connections.

Table 2: Headplate Tubing & Connections

Use	Tubing Type	Length	Size	Connection/Filter
Addition 1	C-FLEX®	20" (500mm)	⅛" ID x ¼ " OD (3.18mm ID x 6.4 mm OD)	⅛-inch Luer-Lok™ connect
Addition 2	C-FLEX®	28" (700mm)	¼" ID x ⅜ " OD (6.4mm ID x 9.6 mm OD)	¼-inch CPC quick-connect
Addition 3	C-FLEX®	28" (700mm)	¼" ID x ⅜ " OD (6.4mm ID x 9.6 mm OD)	¼-inch CPC quick-connect
Sample	C-FLEX®	20" (500mm)	⅛" ID x ¼ " OD (3.18mm ID x 6.4 mm OD)	CLAVE® NeedleFree connector
Harvest	C-FLEX®	28" (700mm)	¼" ID x ⅜ " OD (6.4mm ID x 9.6 mm OD)	¼-inch CPC quick-connect
Exhaust	Silicone (platinum-cured)	8" (200mm)	⅜" ID x ½" OD (9.5mm ID x 12.7 mm OD)	0.2µm filter
Sparge	Silicone (platinum-cured)	2" (50mm)	1/16" ID x ⅛" OD (1.59mm ID x 3.18 mm OD)	0.2µm filter
Overlay	Silicone (platinum-cured)	2" (50mm)	1/16" ID x ⅛" OD (1.59mm ID x 3.18 mm OD)	0.2µm filter

4.6.3 Heat Blanket & Exhaust Tube Heat Blanket

As soon as you unpack the vessel from its protective covering, wrap it with the heat blanket. There are two different heat blankets, one for each vessel size. If you have both vessel sizes, be sure to use the correct blanket for each. Wrap the blanket around the vessel, securing it using the Velcro straps. **Be sure to position the hole in the blanket away from the pH sensor tube** to minimize its exposure to light.

CAUTION!

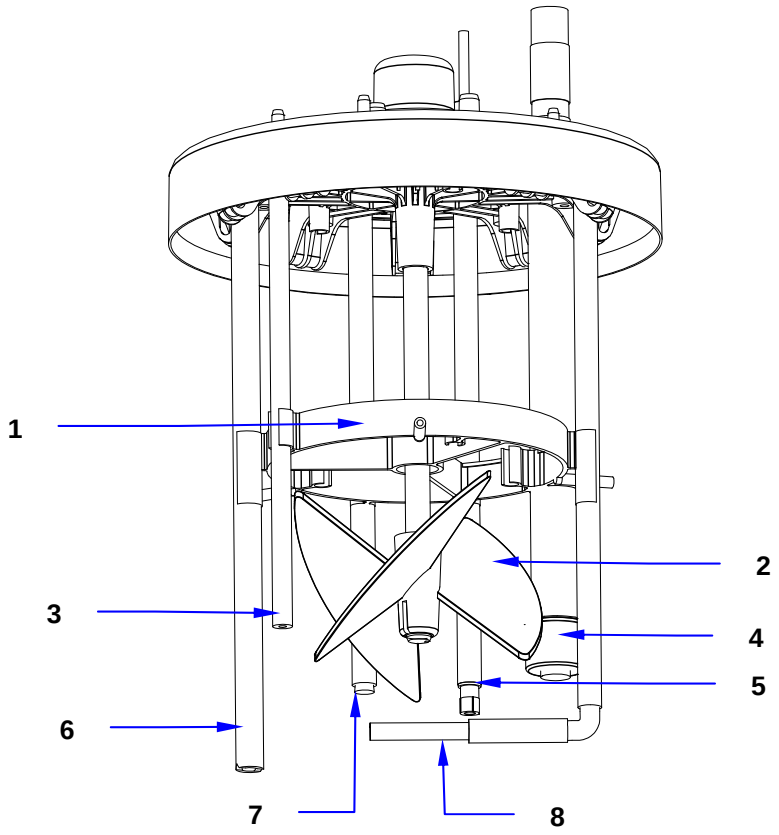
Never turn on the heat blanket or exhaust tube heat blanket without first plugging in the RTD and inserting it into the thermowell inside the vessel (*see Section 4.6.10*).

Attach the exhaust tube heater by wrapping it around the exhaust tube (*see Figure 3 for reference*) and bracing it with the support rod. **The exhaust heater should always be on when gas is flowing into the vessel.**

4.6.4 Internal Components

As illustrated in Figure 3 and in Figure 5 below, the vessel comes completely assembled and sealed for sterility.

Figure 5: Internal Vessel Components



1	Retaining ring (for support)	5	pH tube
2	Pitched blade impeller (upflow)	6	Harvest tube
3	Sample tube	7	RTD thermowell
4	DO tube with silicone cap	8	Porous (7-12µ) sparge

4.6.5 Inspect the pH Probe

Inspect the pH probe for possible shipping damage. When the probe is plugged in and the control cabinet is powered on, a purple light should pulse from the tip of the probe.

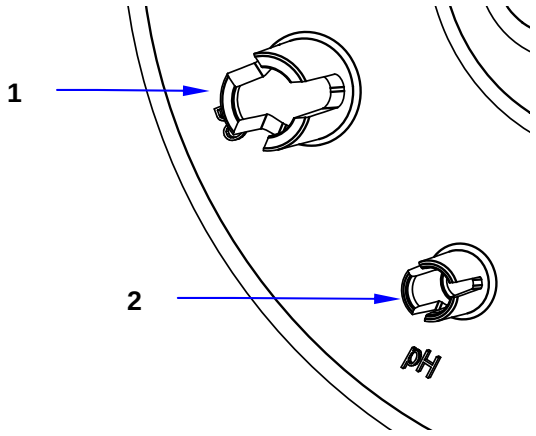
4.6.6 Install the pH Probe

1. Make sure the probe cable is **not** connected to the control cabinet. To protect the sensor from excessive use, attach the cable just prior to calibration (*see Section 7.1*).

2. Gently insert the probe into its headplate port (*see Figures 4a, 4b & 5 on the previous pages for reference, and Figure 5a below*).

NOTE:
The pH port is fluted and the fit is snug, so gently turn the probe as you press it into the port to avoid breakage. When the probe is fully inserted, it becomes difficult to remove: this ensures that the probe remains secure during the culture run.

Figure 5a: pH & DO Headplate Ports (close-up)



1	DO port	2	pH port

4.6.7 Inspecting the DO Probe

Inspect the probe for possible shipping damage; immediately report any damage you may observe to the New Brunswick Scientific Service Department.

Remove the protective cap from the electrode end. The membrane is delicate and care must be exercised to prevent accidental damage. **Never rest the probe on its membrane.**

4.6.8 DO Probe Preparation

Default P & I (proportional & integral) gains are preset at the factory. **It is strongly recommended that you maintain the factory-set parameters.** Nevertheless, P & I gains for the DO loop can be modified by an experienced operator, using the touchpad on the front of the control cabinet.

Whether you choose to use (as recommended) the factory-set values or to alter them, it is highly unlikely that you will ever need to re-set or change them unless you change your process. Even if the power fails during a run, the P & I values (pre-set if you do not change them, or your settings when you do) are stored in memory and should still be in effect when the power is restored. *For details regarding P & I settings, see Sections 22.4 & 22.5.*

It is always prudent to check these values at the beginning of a run.

4.6.9 Install DO Probe

1. Wear protective gloves to protect yourself in case of accidental breakage.
2. Gently insert the probe into its port (*see Figures 4a, 4b, 5 & 5a on the previous pages for reference*).

NOTE:
The DO port is fluted and the fit is snug, so gently turn the probe as you press it into the port to avoid breakage. When the probe is fully inserted, it becomes difficult to remove: this ensures that the probe remains secure during the culture run.

4.6.10 Install Temperature (RTD) Probe

1. Insert the RTD temperature probe (*with reference to Figures 4a, 4b & 5 for location*) into its port, making sure the probe extends all the way to the bottom.
2. Attach the RTD cable to the **Temperature** connector on the control cabinet (*see Figure 2*).

4.6.11 Install Vessel

Position the vessel next to the control cabinet, in the rounded cut-out designed for vessel placement between pumps and connectors.

NOTE:
Remove (puill off) the plastic clips that immobilized the scale during shipping.

If you are using the optional scale, place it under the vessel.

4.6.12 Install Motor Assembly

1. Position the motor assembly on top of the coupling, using the locating notch to orient it properly.
2. Connect the motor cable to the receptacle on the face of the control cabinet.

4.7 Main Power Switch

The main power switch is located on the lower righthand side of the control cabinet (*see Figure 1a,* Front View).

CAUTION!

Before turning on the main power switch, make sure that the power cord is properly connected to the control cabinet and plugged into a suitable power outlet.

4.8 Optional BioCommand *Software*

If you are using NBS supervisory software, be sure to consult your *BioCommand* user's manual for installation and start-up instructions in addition to the general instructions provided below.

A 25-pin RS232/422 Modbus com port is provided on the rear panel of the control cabinet (*see Figure 6*) to connect the CelliGen BLU to a supervisory host computer. Communications to *BioCommand* software are via an optional RS-232 interface cable:

1. Connect the 25-pin end of the RS-232 cable to the *Modbus* port, and ensure that the connection is secure.
2. Hand tighten the thumbscrews.
3. Refer to the *BioCommand* user's guide for instructions on connecting the RS-232 interface cable to the supervisory host computer.

4.9 Inputs/Outputs for Ancillary Devices

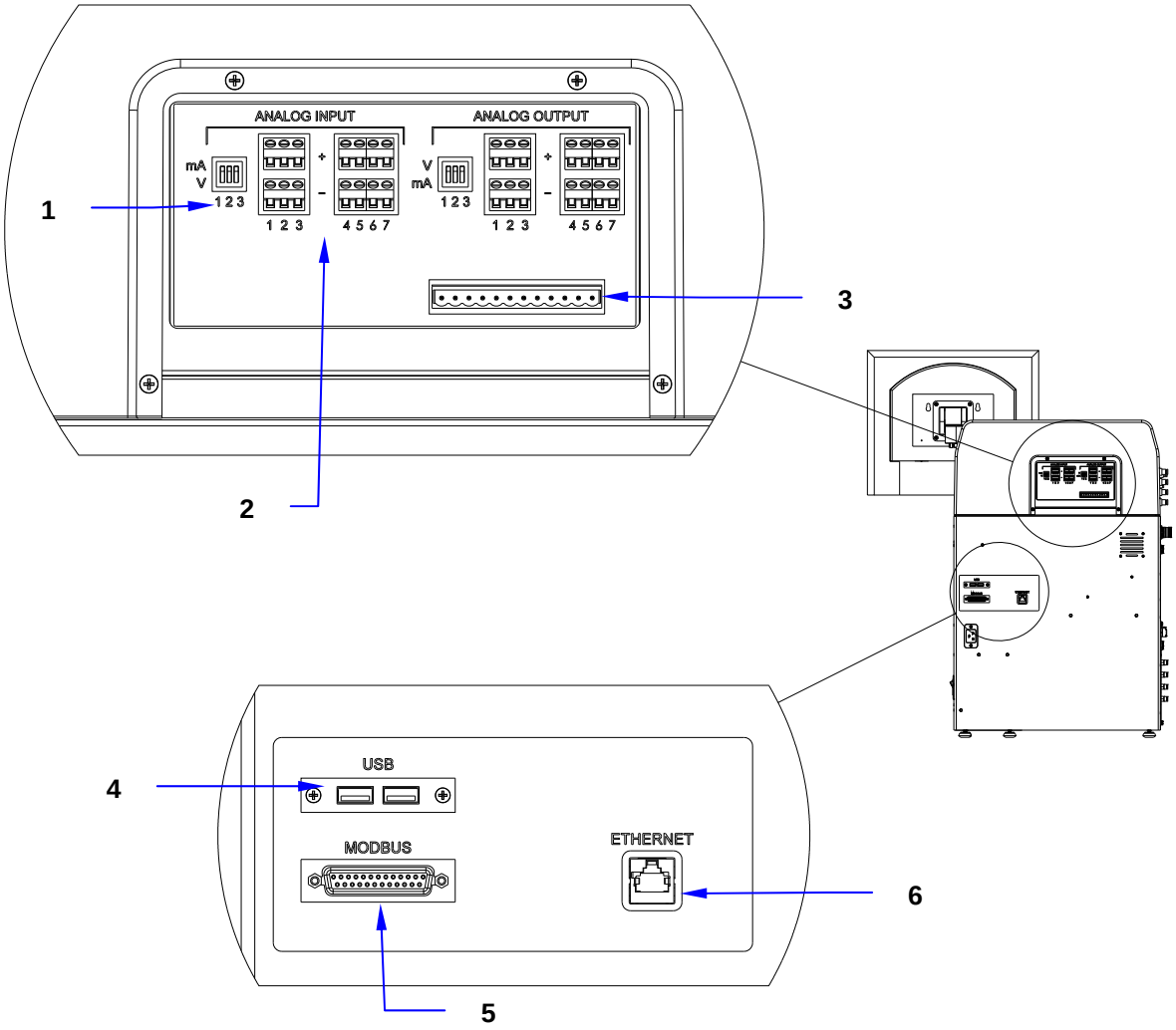
Each thermal mass flow controller (TMFC) uses one 0-5V analog input and one 0-5V analog output port.

Additional analog input and output ports are available on the control cabinet rear panel (*see Figure 5*) for the connection of analog ancillary devices such as additional pumps, gas analyzers and glucose analyzers. After the inputs are connected to the control cabinet, the collected information will be viewed and controlled via the touchscreen display.

Three of these additional analog input and output ports have dip switches to allow selection of either 4-20MA or 0-5V. The other four are 0-5V dedicated.

Two USB serial ports are available on the control cabinet rear panel (*see Figure 6*) for the connection of serial ancillary devices such as scales for vessel and addition bottles. You can connect a box with eight serial (RS232) inputs and outputs to one USB port to allow you to connect and control up to eight scales or other ancillary equipment.

Figure 6: Inputs & Outputs for Ancillary Equipment

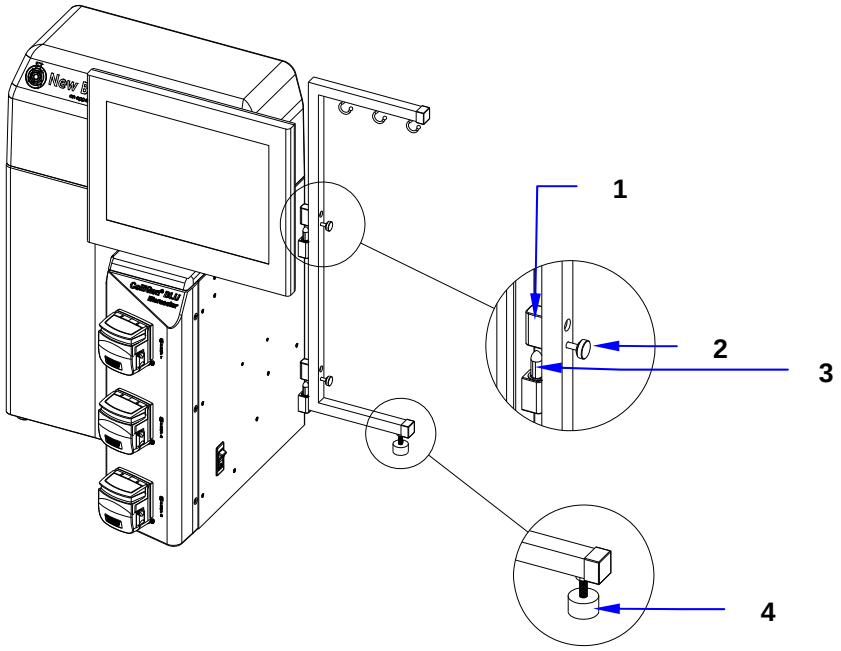


1	Dip switches 1-3, to toggle from 4-20mA (up) to 0-5V (down) for analog input (and output)	4	USB connections are provided for updates or data export.
2	These switches 1-3 can be set to 4-30 mA or 0-5V; switches 4-7 are for 0-5V only.	5	The MODBUS connection is provided for the use of NBS *BioCommand* (see Section 4.8).
3	Capped as standard (not used at this time).	6	Currently not available for use; for future expansion.

4.10 Install the Bag Hanger

If you plan to use the bag hanger provided with the system, install it (*with reference to Figure 6a on the following page*) on the two hinge pins located on the righthand side of the controller:

Figure 6a: Bag Hanger



1	Bracket hinge	3	Hinge pin on controller
2	Thumbscrew	4	Adjustable foot

1. With the three hooks at the top of the bracket and the adjustable foot at the bottom, orient the long side of the bracket against the side of the controller, sliding its two hinges onto the pins that are mounted on the controller.
2. The bracket swings freely on the hinges unless you choose to fix it in place at the desired angle by tightening its two thumbscrews against the controller.
3. Be sure to tighten the adjustable foot to secure the bracket on the benchtop before you add any contents to the bag(s) you hang there.
4. As needed, feed the tubing from the bag(s) into the dedicated pump(s) and aseptically attach the tubing to the appropriate port connection (addition, harvest or sampler).

CAUTION!

Do not allow the combined weight of the bags and their contents to exceed 15 kg (33 lb).

5 SPECIFICATIONS

CelliGen BLU System			
Cell Culture Vessel			
	Working Volume	3.75L	10.5L
	Total Volume	5.0L	14.0L
Controller	**Control Station**	Controls up to 32 control loops; stores 10 recipes & 8 process variables for trend graphing. Includes an industrial touchscreen monitor/user interface, 3 built-in pumps & connectors for all utilities & communications signals.	
	Touchscreen Interface/Display	15-inch industrial monitor is standard with the Control Station.	
Temperature	**Indication**	Digital display in 0.1°C increments	
	Range	From 5°C above ambient temperature to 40°C	
	Control	P&I for heating and cooling: heating via the Heat Blanket and cooling via ambient temperature	
	Sensor	Platinum RTD probe	
Agitation	**Drive**	Permanent magnet motor with high torque input.	
	Indication	Digital display in 1 RPM increments.	
	Range	25-200 RPM	
	Control	PI-controlled	
	Sensor	Optical photoplastic disc 500 lines/rev with quadrature output.	
	Impellers	Pitched blade	
Exhaust	**Filter**	0.2μm disposable filter	
	Tube	Heat blanketed	
Aeration	**4-Gas System**	Up to 4 gases, including air, N_2, CO_2 & O_2, delivered to sparger	
	Sparger	Porous Microsparger (7-12μ)	
	Inlet Filter	0.2μm disposable filter	
	N_2 Gas	For calibration of DO probe	
pH	**Indication**	Digital display in 0.01 pH increments	
	Range	6-8 pH	
	Control	P&I	
	Sensor	Non-invasive optical pH probe	
DO	**Indication**	Digital display in 0.1% increments	
	Range	0-200%	
	Control	3- or 4-gas P&I control	
	Sensor	Non-invasive polargraphic probe	

...continued...

CelliGen BLU System				
Pumps[1]	**Pump 1**	Assignable peristaltic pump Fixed speed (14 RPM) or variable duty cycle Available control modes: Off, On, Prime.		
	Pumps 2 & 3	Assignable peristaltic pumps Fixed speed (109 RPM) or variable duty cycle Available control modes: Off, On, Prime.		
Utilities	**Gas**	6 PSIG maximum		
Electrical Requirements	**100-120VAC**	50/60 Hertz	Single phase	15 Amps
	200-240VAC	50/60 Hertz	Single phase	15 Amps
Net Weight	**Control Station**	88 lbs. (40 kg) with touchscreen		
	Touchscreen	15 lbs. (6.8 kg)		
	Vessel empty, without motor[2]	**5.0L**		**14L**
		2.9 lbs/ (1.3 kg)		3.9 lbs (1.8 kg)
Overall Dimensions with Touchscreen		25 in. wide X 24 in. deep X 34 in. high (63 cm W X 61 cm D X 86 cm H)		
Overall Dimensions without Touchscreen		18 in. wide X 24 in. deep X 28 in. high (46 cm W X 61 cm D X 71 cm H)		
External Computer Connections		Port supplied for remote connection of interface computer		
***BioCommand* Connections**		Port supplied for connection of *BioCommand* supervisory host computer.		
Fuses		One 5A glass tube, fast-acting fuse		
Regulatory Compliance		*See Section 5.1*		
Ambient Operating Conditions		10-30°C, up to 80% relative humidity, non-condensing		

[1] See Table 8 (*Section 13.3*) for pump flow rates according to tubing size
[2] Vessel weight does not include probes, exhaust condenser or other options.

5.1 Validation Documentation

All pertinent validation documents are available online at www.nbsc.com/BLU . With the vessel's lot number in hand (see bottom of vessel), double click on the PDF icon for CelliGen BLU Validation Guide in the lefthand column.

5.2 Certifications

The CelliGen BLU has been tested to ETL standards, to comply with these safety standards:

- UL 61010A-1
- UL 61010A-2-010
- CAN/CSA-C22.2 No. 1010.1
- CAN/CSA-C22.2 No. 1010.2.010

As attested in the CE Declaration of Conformity reproduced on the following page, they also conform to the appropriate CE standards.

New Brunswick
an eppendorf company

CE CE

DECLARATION OF CONFORMITY

New Brunswick Scientific hereby declares that the product(s) listed below conform to the European Union directive and standards identified in this declaration.

Product(s)

CelliGen BLU

EU Directive(s)

Low Voltage (2006/95/EC)
Electromagnetic Compatibility (2004/108/EC)

Standard(s)

EN61010-1
EN61010-2-010
EN55011
EN61000-3-2
EN61000-3-3
EN61000-4-2
EN61000-4-3
EN61000-4-4
EN61000-4-5
EN61000-4-6
EN61000-4-11

The conformity assessment procedures were performed at the following notified body:

- **Intertek Testing Services, 41 Plymouth Street, Fairfield, NJ 07004; Advanced Compliance Laboratory, 6 Randolph Way, Hillsborough, NJ, 08844; New Brunswick Scientific, 44 Talmadge RD, Edison, NJ 08818.**

The technical documentation relevant to the above equipment will be held at:
New Brunswick Scientific Company
PO Box 4005
44 Talmadge Road
Edison, New Jersey 08818-4005 U.S.A
Tel. (732) 287-1200
Fax. (732) 287-4222

Joe Viola
Director of QA RA

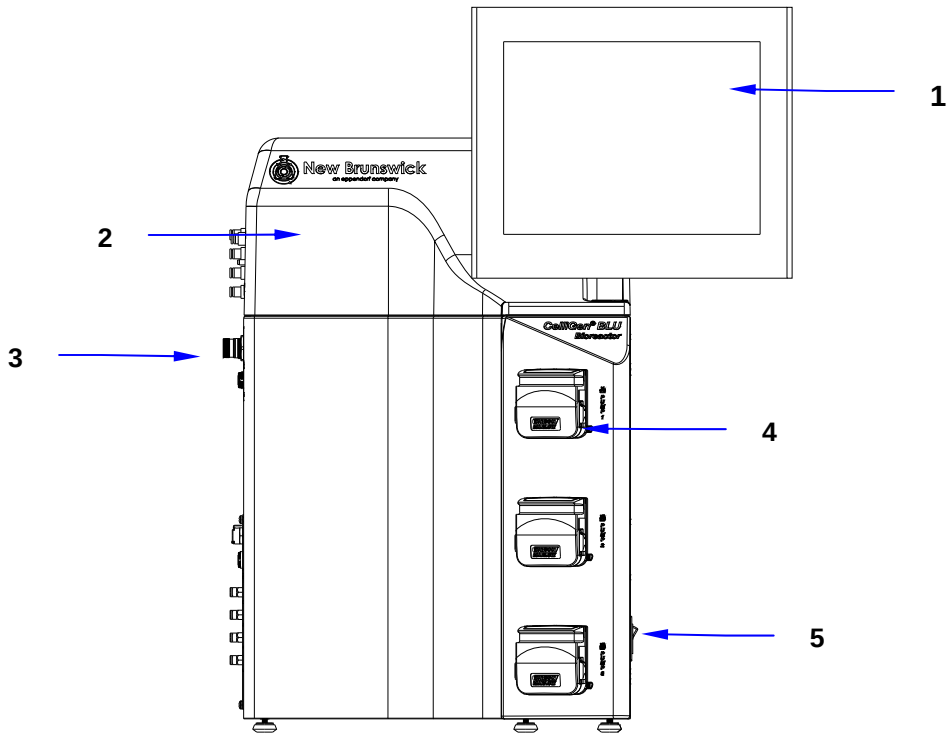
12/7/2010
Date

6 OPERATING CONTROLS

6.1 Touchscreen

Your primary interface with the CelliGen BLU is the touchscreen on the control cabinet:

Figure 7: Touchscreen



1	Touchscreen display	4	Pumps
2	Control cabinet	5	ON/OFF power switch
3	Service Connections (see Section 4.5, Figure 2)		

6.2 Display Screens

6.2.1 Touchscreen Calibration

The first time you power up, you will be prompted to calibrate the screen to your touch. Follow the onscreen instructions to touch the target each time it appears. Usually you will be prompted to touch the four corners of the screen, twice in succession. (See important **NOTE** on the following page.)

NOTE:
For optimal results, be sure to stand or sit in the position from which you are most likely to work. Height and angle of reach will affect calibration.

6.2.2 Start-Up Screen

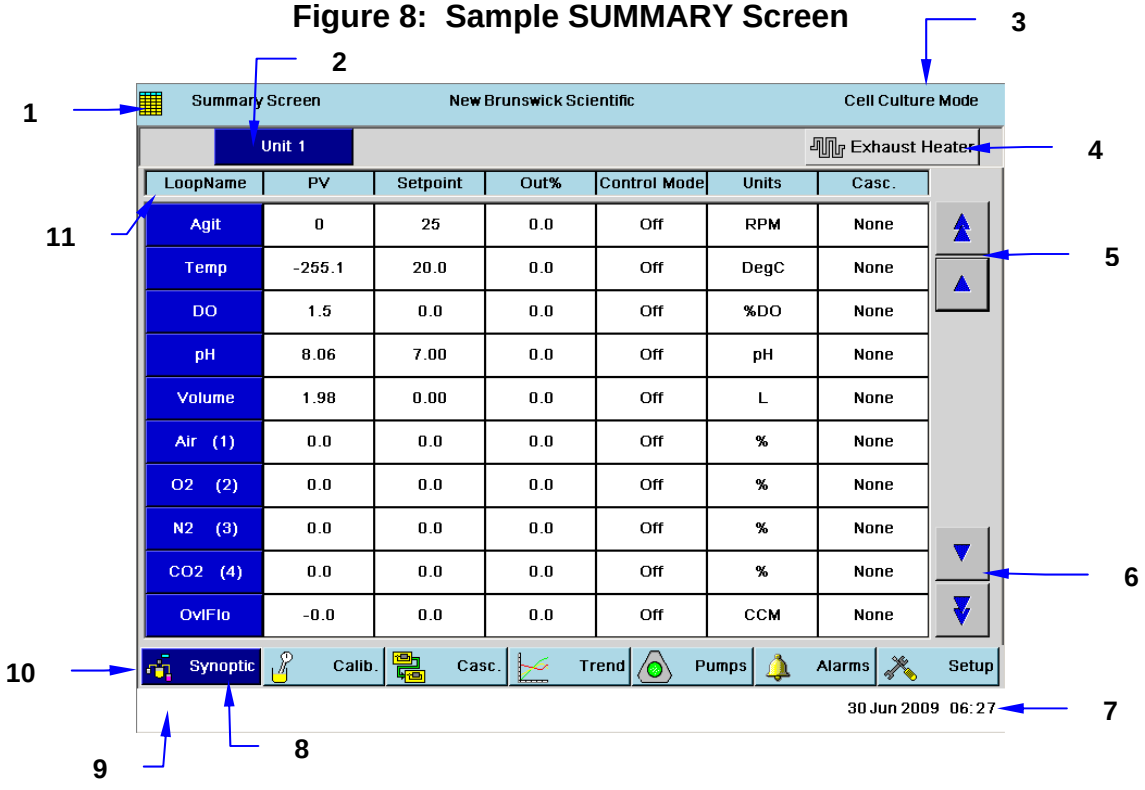
The Start-Up screen, which tells you which operating software version is installed in your CelliGen BLU, is first screen you see each time you turn on the power, if you have already calibrated the touchscreen (*see Section 6.2.1*).

This screen remains in view for a few seconds, then it is replaced by the **SUMMARY** screen.

6.2.3 Summary Screen

The **SUMMARY** screen (*see Figure 8*) is command central; it puts as many as 32 loops at your fingertips.

Figure 8: Sample SUMMARY Screen



1	Screen Name and Icon	8	**NOTE:** The dark blue button usually represents the screen being displayed. Here it shows a new screen, SYNOPTIC, that is accessible from this screen (see Section 6.2.4 for details).
2	Unit Tab		
3	Operating Mode		
4	Exhaust Heater button	9	Any ALARMS will appear in this space.
5	Scroll Up buttons	10	Screen Access buttons
6	Scroll Down buttons	11	Your CelliGen BLU comes with pre-assigned loop names. As you add more, use the Scroll Down buttons to see them.
7	Current Date & Time		

Table 3 below identifies the other interactive features of the **SUMMARY** screen:

Table 3: SUMMARY Screen Features

Parameter Column	Description
LoopName	The system comes with standard factory-assigned control loops (e.g., Agitation, Temperature, pH, DO, etc.) for the bioreactor. There are also unassigned loops available, to be named and set up by the user when adding external equipment, for a maximum total of 32 loops.
PV	Process Variable: here the display reflects the current value for each loop, in comparison to its setpoint (displayed in the next column).
Setpoint	The current setpoint (default or user-set) for each loop.
Out%	The current percent output for each loop. This is an automatic control function to maintain current readings within the setpoint tolerance range.
Control Mode	Depending on the loop, the control mode may be Off, Auto, Manual, On, or 4 Gas.
Unit (of measure)	This is the unit of measure used for the PV and Setpoint.
Cascade	If any cascades have been programmed, they will be displayed here.
Summary[1]	This screen is command central; it shows all your loops, their current readings, setpoints and what has been programmed for them.
Synoptic[1]	This screen is a graphical alternative to the SUMMARY screen. It shows your loops, their current readings and their setpoints. It also displays the current state of the fixed speed pumps, level probes and process valves.
Calibration	This screen allows you to calibrate the DO probe, the pH probe and vessel volume.
Cascade	A cascade is a control function that uses the output of one loop to influence the action and output of one or more other loop(s). This screen allows you to set up cascades, to view current settings, and to make changes to those settings.
Trend	This screen allows you to set the parameters for plotting trend graphs and to view the graphs that track the activity of the selected loops over a certain period of time.
Pumps	This screen gives you access to the Pump Gauges screen, where the three pump gauges are displayed, providing both current readings and the opportunity to change pump settings.
Alarms	In this screen you can turn alarms on and off, read the alarm history and acknowledge any alarm.
Setup	This screen allows you to load & save recipes and to make changes to your system settings, hardware setup & controller setup.

1: see the following page

…continued…

Other Buttons	Description
Exhaust Heater[2]	Press this button to turn the exhaust heater on or off when it is connected to the control station. The button is green when the heater is on.
Scroll Up	Press this button to scroll upwards, one loop at a time.
Scroll Down	Press this button to scroll downwards, one loop at a time.

1 The far left navigation button at the bottom of all main screens is a toggle between the **SUMMARY** and the **SYNOPTIC** screens. When viewing one, the button will be labeled for the other. Upon leaving either for one of the other screens, the default selection shown on the button will be the most recently visited of the two. That is, if you leave the **SUMMARY** screen to view the **TREND** screen, for example, the far left button will be labeled **SUMMARY**.

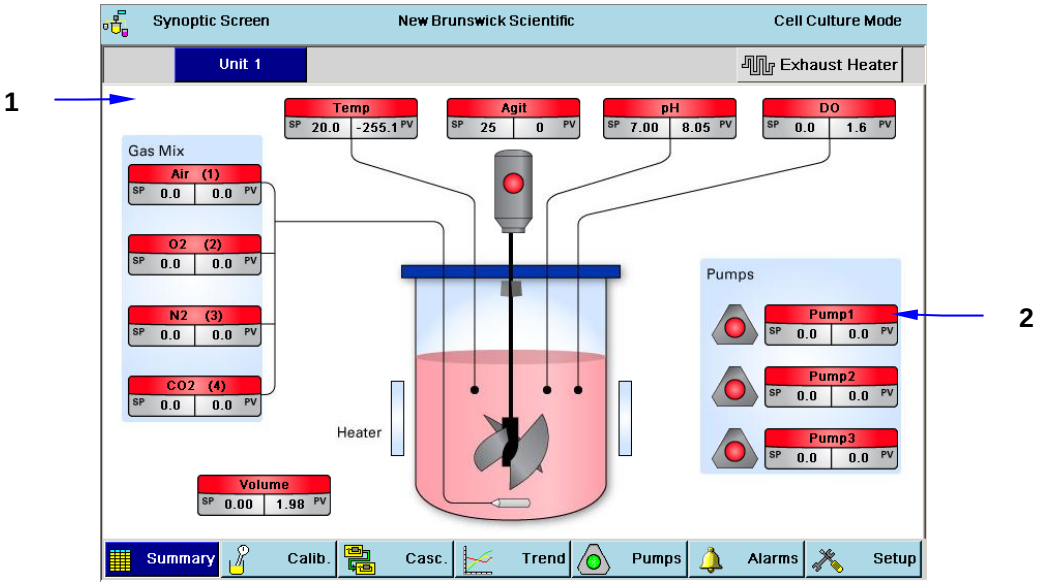
2 It is very important to be sure the exhaust heater is on when you are actively growing cells, to reduce the risk of clogging the exhaust filter.

6.2.4 Synoptic Screen

From any main screen, press the far left **SYNOPTIC** button to open the **SYNOPTIC** screen (*see Figure 9 on the following page*). If the far left button says **SUMMARY**, press it to open the **SUMMARY** screen. The button will now be labeled **SYNOPTIC**; press it again.

This screen provides a visual representation of all the loops, their settings and current process values—the sample screen below may not be exactly like the screen you see; it depends on your system's options. This screen provides all the functionality of the **SUMMARY** screen with the exception of the ability to add loops.

Figure 9: Sample Synoptic Screen

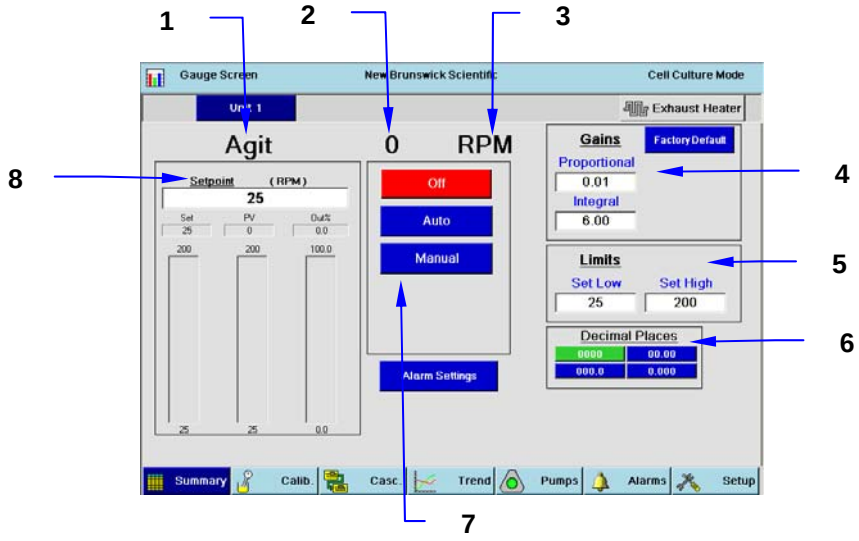


1	LOOPS: Each gauge indicates setpoint (SP) and process variable (PV). Title color indicate the loop's status: Red = OFF, Green = ON and Blue = MANUAL. Touch the loop gauge in this screen to open the full loop gauge screen (see Section 6.2.5).
2	PUMPS: Each gauge indicates setpoint (SP) and process variable (PV). Pump icon & gauge title color indicates that pump's status: Red = OFF and Green = ON.

6.2.5 Gauge Screens

Every loop has its own gauge screen. To access it, in the **SUMMARY** screen, touch the screen inside the appropriate blue box in the **LoopName** column. Your touch will open that loop's **GAUGE** screen (*see Figure 10, on the following page*).

Figure 10: Sample GAUGE Screen



1	Loop Name
2	Process Variable (present value)
3	Units: the action of this loop, Agitation, is measured in RPM.
4	P&I values
5	Limits: Here you adjust the high & low settings for this specific loop. When adjusted, the scaling for the gauge (on the left of the screen) will also be adjusted to reflect the high & low limits selected.
6	Decimal Places: Press the appropriate button to display values with 0, 1, 2 or 3 decimal places.
7	Control Mode
8	Setpoint (and Units of measurement)

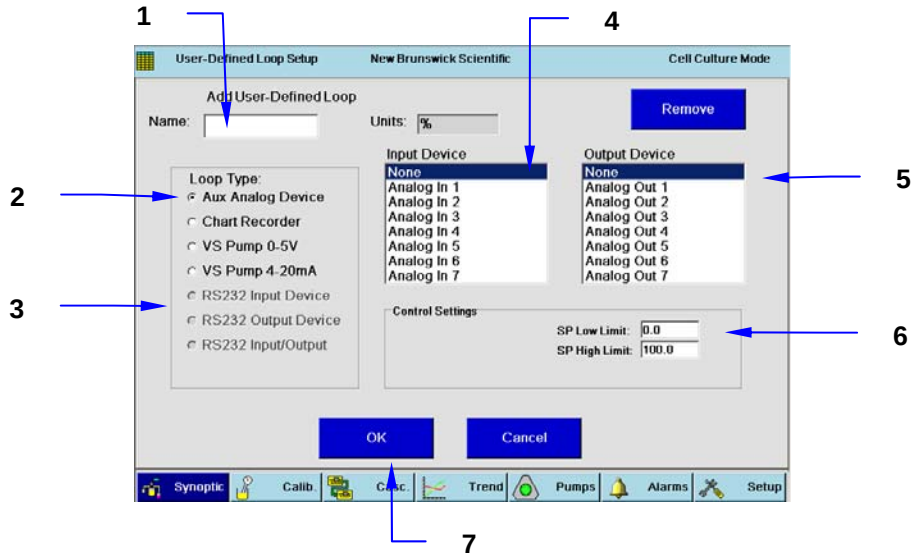
6.2.6 Adding Loops

The CelliGen BLU comes to you with standard factory-assigned loops and the possibility to add more loops, which are related to external auxiliary equipment, added via standard analog and optional serial (RS232) inputs/ outputs located on the rear panel.

To add a new loop:

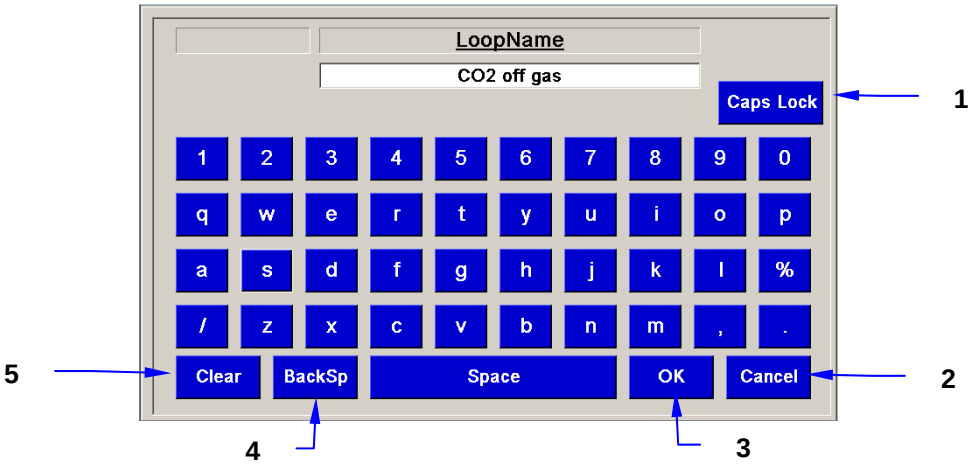
1. Scroll down in the **SUMMARY** screen beyond the last pre-assigned loop, and press on a blank **LoopName** box.
2. The **Add User-Defined Loop** screen will open (*see Figure 11 on the following page*).

Figure 11: Add User-Defined Loop Screen



1	Step 3: Press here and use the LoopName Touchpad (*see Figure 12*) to name the loop.
2	Step 4: Press the appropriate option button. The corresponding Unit of measurement will automatically appear (% in this sample screen).
3	NOTE: Options are not available (grey) if the system does not detect their presence.
4	Step 5: Press the appropriate Input Device designation.
5	Step 6: Press the appropriate Output Device designation.
6	Step 7: Input the desired Control Settings (Setpoint low & high limits).
7	Step 8: After making all of your selections, press the OK button to save them.

Figure 12: LoopName Touchpad



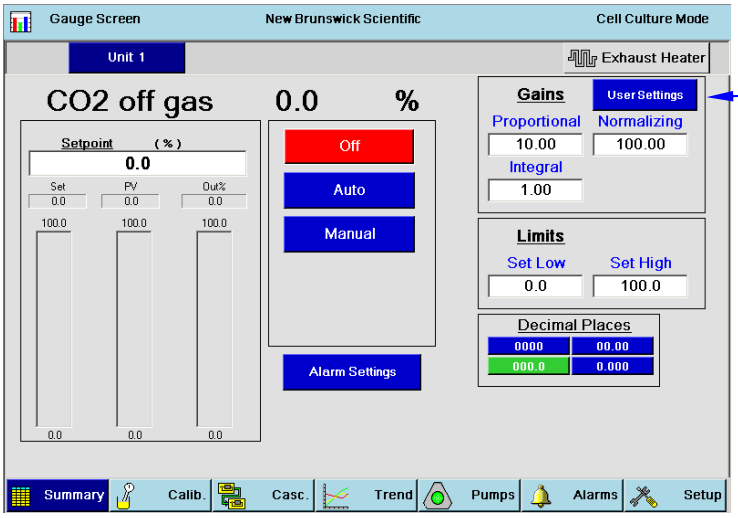
1	Press **Caps Lock** to shift to CAPITAL letters. Press it again to shift back to lower case.
2	Press **Cancel** to return to the Gauge screen *without* saving work done with the touchpad.
3	Press **OK** to return to the Gauge screen, *saving* the work done with the touchpad.
4	Press **BackSp** to backspace, cancelling one character at a time.
5	Press **Clear** to clear the LoopName edit box in this touchpad, allowing you to begin again.

6.2.7 Deleting Loops

Only user-added loops can be deleted. If you wish to delete a loop:

1. In the **SUMMARY** screen press the **LoopName** box for the loop you wish to delete.
2. In the loop's **GAUGE** screen, and **if this is not a pump control loop**, press the **UserSettings** button (*see Figure 13*):

Figure 13: Deleting a Control Loop

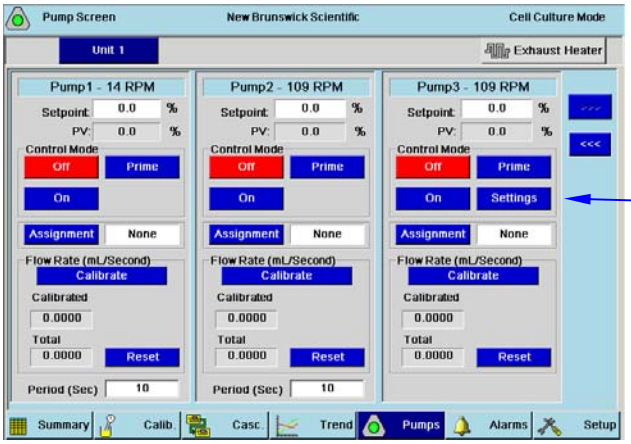


1	Press here to open the Add User-Defined Loop screen.

3. In the **Add User-Defined Loop** screen, press the **Remove** button in the upper righthand corner.

4. **If the loop is a pump:** only optional pumps have a **Settings** button that provides access to their **Remove** button (*see Figure 14 on the following page*).

Figure 14: Deleting a Pump Control Loop



1	Press the **Settings** button to open the screen where you can press the **Remove** button to delete the pump loop. If there is no **Settings** button in the pump gauge (see Pump1 & Pump2 for example), that pump's control loop cannot be deleted.

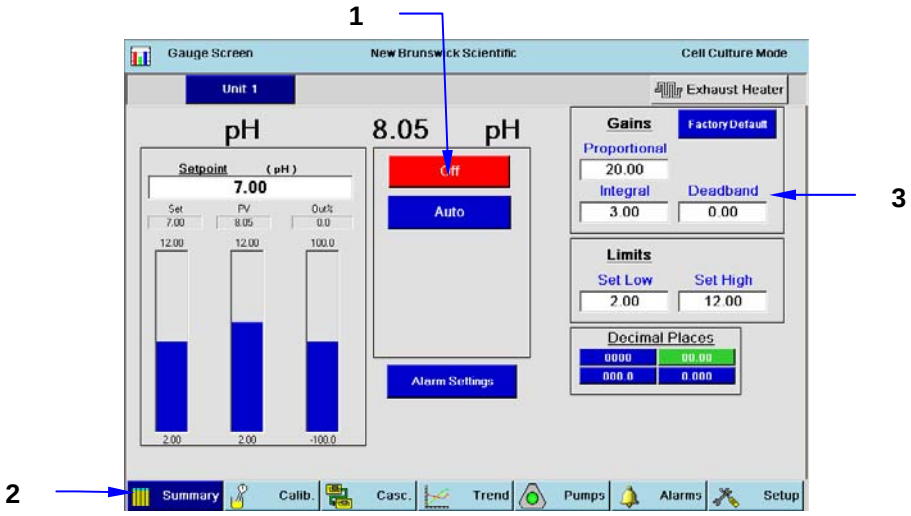
5. When you return to the **SUMMARY** screen, the loop will be deleted.

6.2.8 Selecting Loop Control Modes

Control modes vary according to the loop and process mode. (There are also operating modes for all of the pumps; *see Section 13.2* for details.) To change operating modes for any of the displayed loops, in the **SUMMARY** screen:

1. Press either the **LoopName** or the **Control Mode** box in the row for the appropriate loop to open that loop's **GAUGE** screen (*see Figure 10 on the following page*).

Figure 10: Sample GAUGE Screen



1	Step 2: Press the button that corresponds to the desired Operating Mode.
2	Step 3: To save the new operating mode and return to the SUMMARY screen, press the **SUMMARY** button.
3	Deadband is a user-definable pH value within which, above or below the setpoint, no response will be triggered.

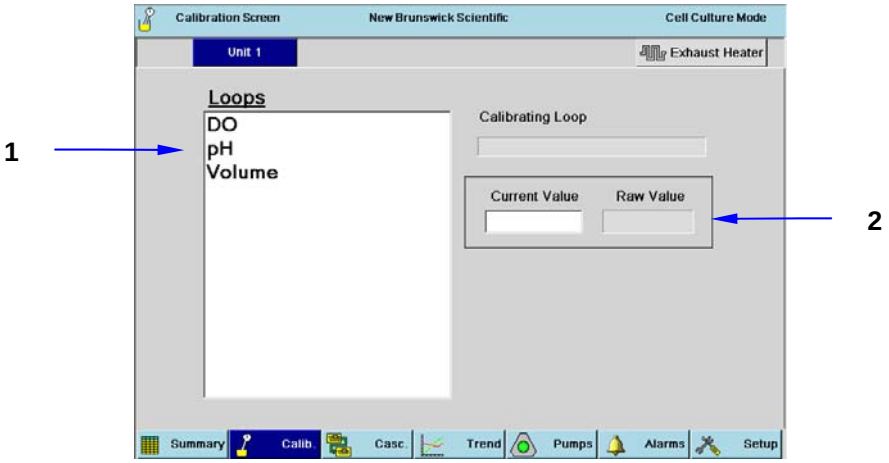
NOTE:

Sections 6.2.9–6.2.14 introduce you to the other main screens accessible by pressing the blue navigation buttons at the bottom of the screens.

6.2.9 Calibration Screen

This screen is used to calibrate the pH and the DO probes and vessel volume.

Figure 15: Calibration Screen



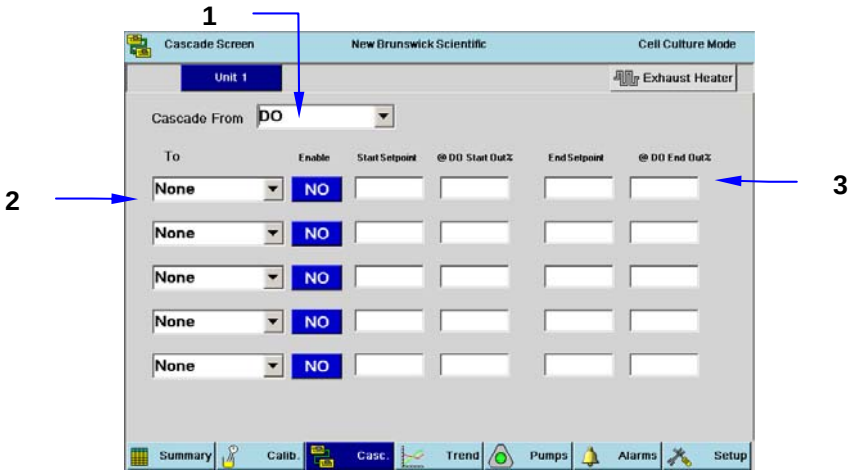
1	Loops available for calibration	2	Input boxes

For details on probe calibration, see Sections 7.1 (pH probe) and 7.3 (DO probe). For details on volume calibration, see Section 7.5.

6.2.10 Cascade Screen

A cascade is a control function that uses the output of one loop to influence the action and output of one or more other loop(s). This screen (*see Figure 16*) allows the user to set up cascades, to view current cascade settings and to change those settings.

Figure 16: Cascade Screen



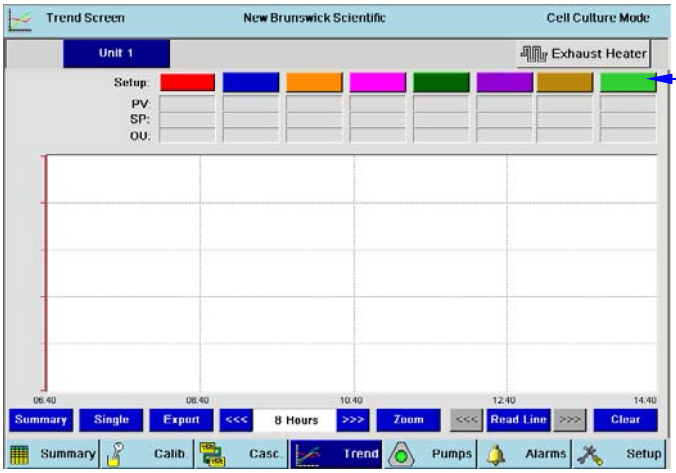
1	Loop being cascaded *from*
2	Loop(s) being cascaded *to*
3	User-definable variables

For details on setting cascades, *see Section 10.1*.

6.2.11 Trend Screen

This screen (*see Figure 17 on the following page*) allows the user to set the parameters for plotting trend graphs and to view the graphs that track the activity of up to 8 selected loops during an entire process run. The data can be exported through the USB port in Excel format to a PC. For details on using the **TREND** screen, see Section 12.

Figure 17: Trend Screen

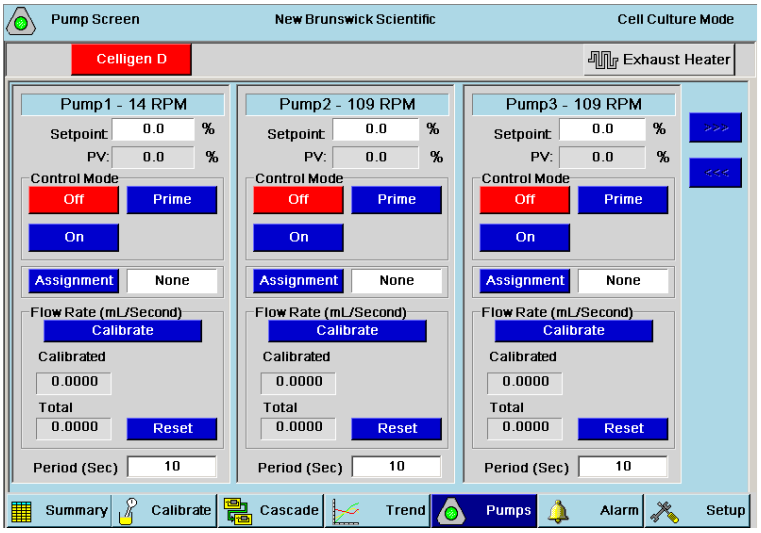


1	The user will assign a tracking color to each loop.

6.2.12 Pumps Screen

This screen (*see Figure 18*) allows the user to access the pump gauges screens, where the three standard pumps (plus any optional pumps) are displayed, providing both current readings and the opportunity to change pump settings. For details on using the **PUMPS** screen, see Section 9.5.

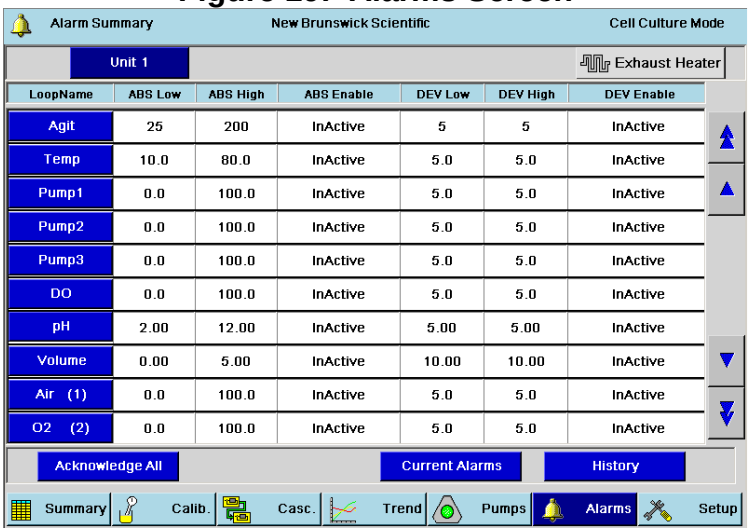
Figure 18: Pumps Screen



6.2.13 Alarms Screen

This screen (*see Figure 19*) allows the user to turn alarms on and off, to read the alarm history and to acknowledge any alarm while it is active. For details on using the **ALARMS** screen, *see Section 14*.

Figure 19: Alarms Screen

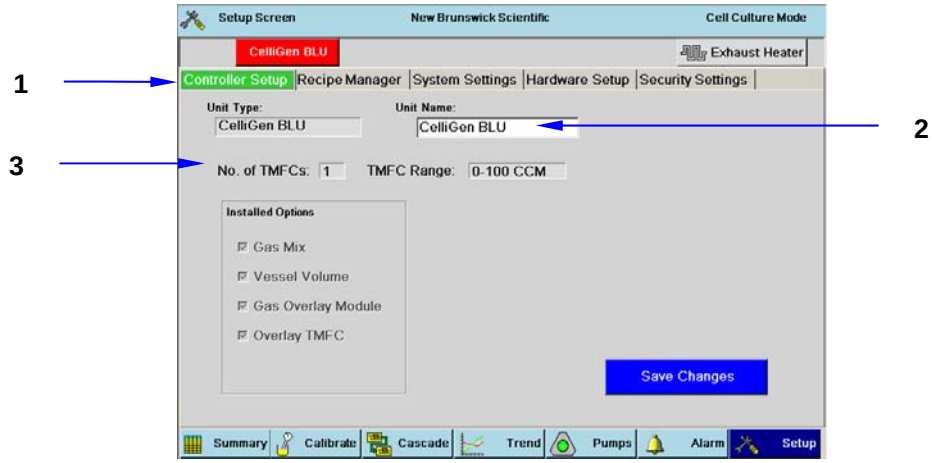

Alarm Summary
New Brunswick Scientific
Cell Culture Mode
Unit 1
Exhaust Heater
LoopName ABS Low ABS High ABS Enable DEV Low DEV High DEV Enable
Agit 25 200 InActive 5 5 InActive
Temp 10.0 80.0 InActive 5.0 5.0 InActive
Pump1 0.0 100.0 InActive 5.0 5.0 InActive
Pump2 0.0 100.0 InActive 5.0 5.0 InActive
Pump3 0.0 100.0 InActive 5.0 5.0 InActive
DO 0.0 100.0 InActive 5.0 5.0 InActive
pH 2.00 12.00 InActive 5.00 5.00 InActive
Volume 0.00 5.00 InActive 10.00 10.00 InActive
Air (1) 0.0 100.0 InActive 5.0 5.0 InActive
O2 (2) 0.0 100.0 InActive 5.0 5.0 InActive
Acknowledge All
Current Alarms
History
Summary
Calib.
Casc.
Trend
Pumps
Alarms
Setup

6.2.14 Setup Screen

This master **SETUP** screen (*see Figures 20a, 20b, 20c & 20d on the following pages*) is actually comprised of four screens, accessed by tabs, which are used to set up the controller, recipe management, system settings and hardware for the CelliGen BLU system. This section will introduce you to those screens and their features. For details on using the **SETUP** screen, including a fifth tab that may be present, see Section 15.

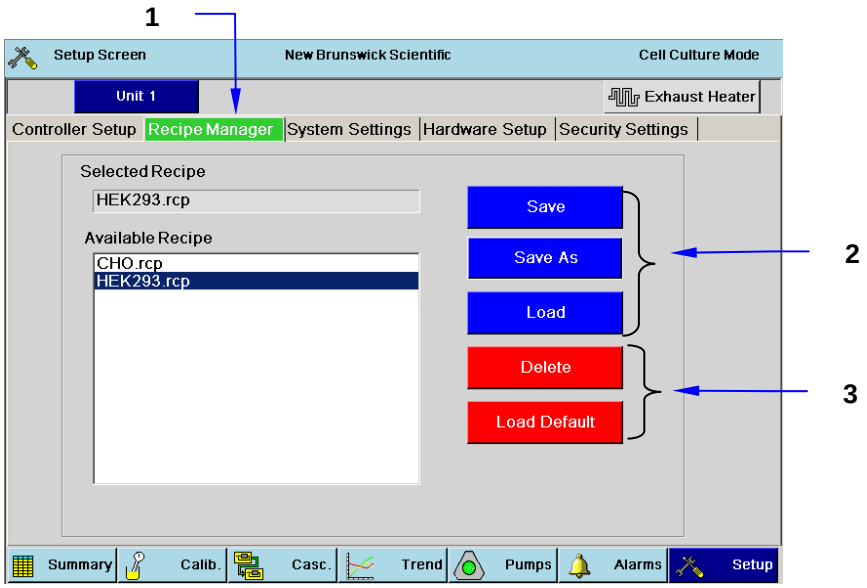
When you press the **SETUP** button, the screen that opens is actually the first tab, the **CONTROLLER SETUP** (*see Figure 20a on the following page*) screen:

Figure 20a: Controller Setup Screen



1	Controller Setup TAB
2	The Unit Name can be user-selected. Press this box, then use the pop-up touchpad to type in the desired name.
3	The TMFC (Thermal Mass Flow Controller) Range and the number of TMFCs (0 means manual gas flow, usually by rotameter) are factory-set.

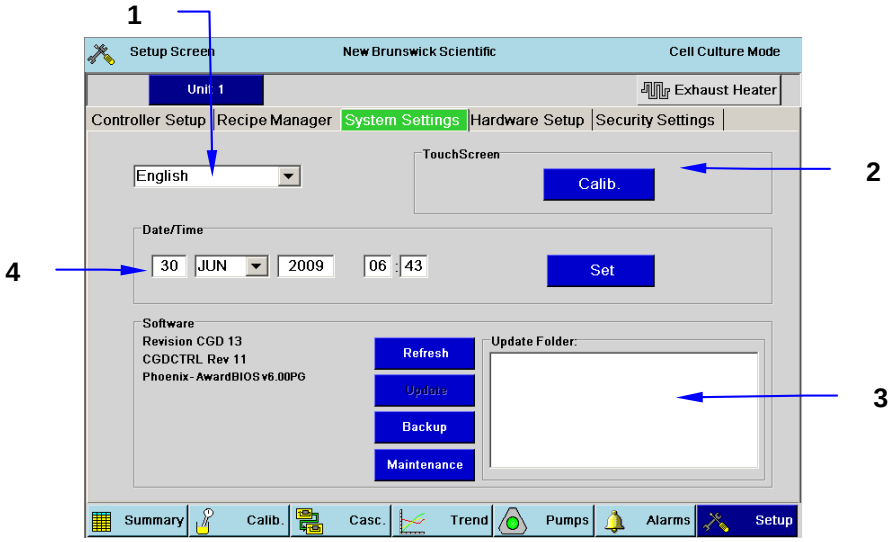
Figure 20b: Recipe Manager Screen



1	Use the Recipe Manager screen to save and load up to 10 recipes.
2	Recipes can be saved and loaded using these top three buttons.
3	The Delete button removes the currently selected recipe; the Load Default button restores factory settings.

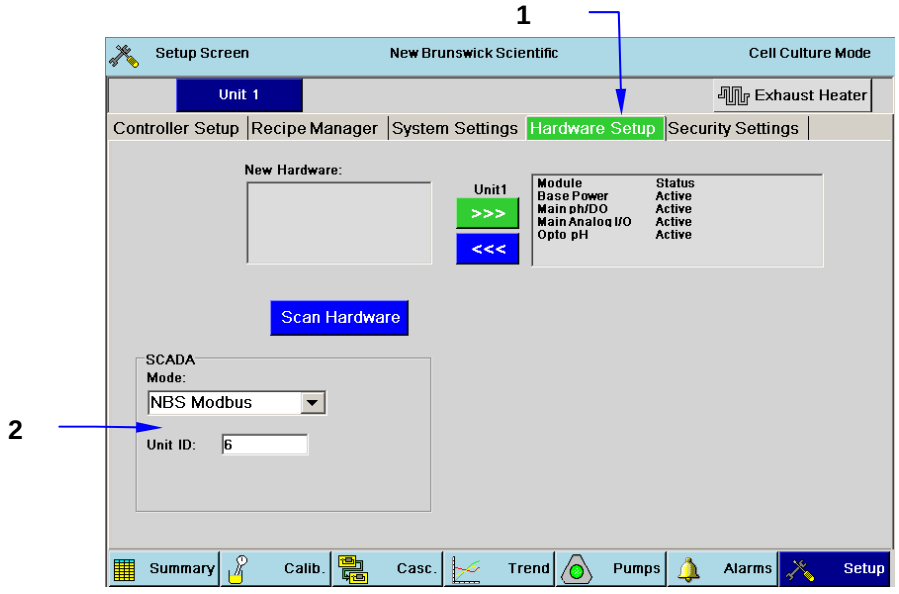
A **recipe** consists of all setpoints, controller settings, control modes, calibration data and cascades set on a system.

Figure 20c: System Settings Screen



1	English is the default language. When other choices (Français, Deutsch, Español) become available, the user will select the language here.
2	Use this pane to calibrate the touchscreen (see Section 6.2.1 for details).
3	Use this pane to view the Software/Firmware version installed, and to update software via the USB port (see Section 15.3.2 for details).
4	Use this pane to change Date and Time (see Section 15.3.1 for details).

Figure 20d: Hardware Setup Screen

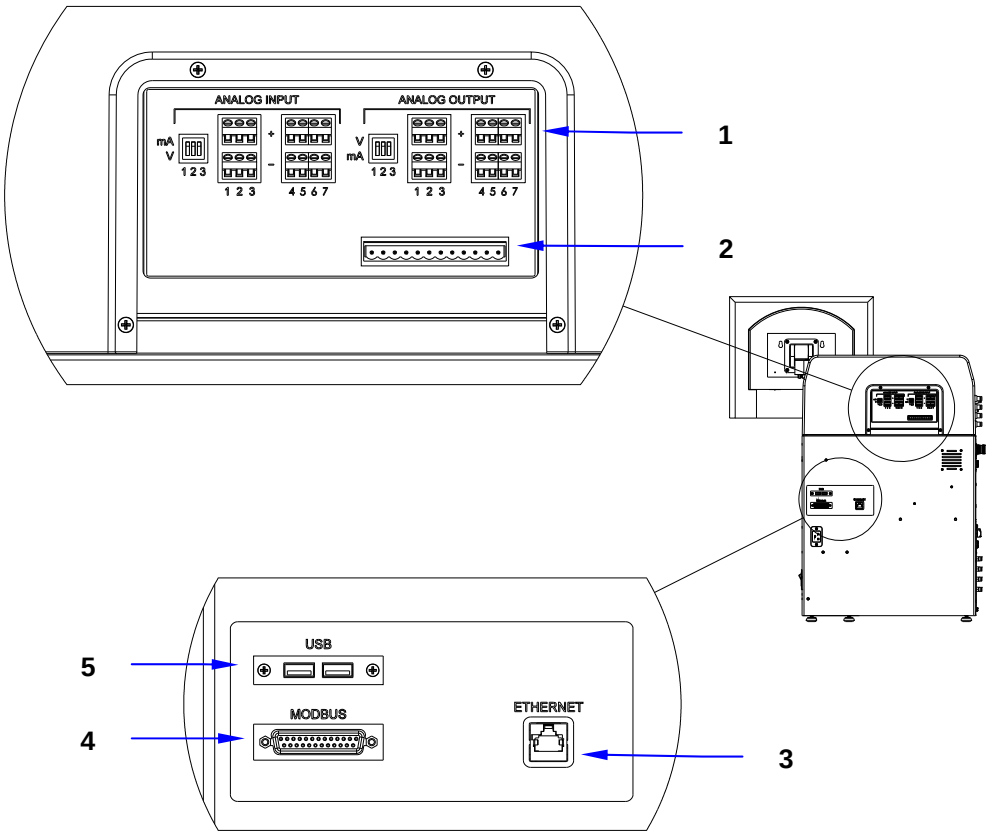


1	Use the Hardware Setup screen to view hardware installed in the system and to set Unit IDs for software.
2	Use this pane to choose software connections.

6.3 RS232/422 Computer Interface

An RS232/422 com port has been provided; there is a 25-pin "D" connector located on the lower rear panel of the control cabinet *(see Figure 21)*. It is labeled **Modbus**.

Figure 21: Control Cabinet Rear Panel



1	Analog Inputs & Outputs are easily accessible
2	Capped as standard: not in use at this time
3	Ethernet port: for future expansion; not in use at this time
4	Modbus port, for use with NBS BioCommand supervisory software
5	2 USB ports

An NBS *BioCommand* advanced supervisory software program is available which will enable the operator to interface with a computer that has a Windows® 2000 (or higher) operating system. With this software, you will be able to establish or change the setpoints for temperature, pH, DO, agitation speed and pump flow rate. You will also be able to read and log the process values of any parameters (temp, pH, DO, air flow, pump flow rate, levels and agitation) that are monitored. The data can also be stored, plotted and, afterwards, transferred to other commonly available programs, to be manipulated and analyzed in various ways.

Table 4 identifies the pin designations for this 25-pin RS232/422 connector:

Table 4: Modbus Com Port Pin Designation

Pin Number	Signal	Comments
1, 4-6, 8-11, 14-20, 22-23	NC	not assigned
2	TXD	RS232 Data Output from bioreactor
3	RXD	RS232 Data Input to bioreactor
7	GND	Ground reference for all signals
12	IRXD+	RS422 paired data input to bioreactor
24	IRXD-	
13	ITXD+	RS422 paired data output from bioreactor
25	ITXD-	
21	IOS	Open selects RS232 Grounded selects RS422

Unless otherwise requested, the baud rate is factory-selected at 19200 (Modbus) and the connector is configured as an RS232 port: i.e., no jumper between pin #7 and pin #21. The factory-set address for the machine is **8**.

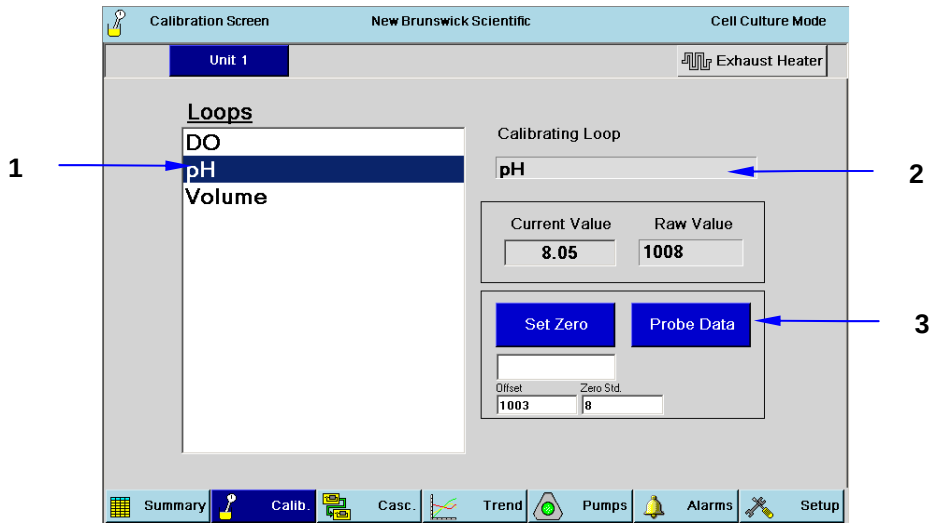
7 PREPARATION & CALIBRATIONS

7.1 pH Calibration

The pH probe provided with this system is a reusable probe, which works in conjunction with a disposable optical pH sensor; the light that blinks at the tip of the probe when it is plugged in to the control cabinet is not dangerous to your vision. To ensure proper calibration of the sensor and to compensate for any discrepancy, **you will need an accurate external pH meter**.

1. Press the **Calib.** Button at the bottom of the **SUMMARY** screen to open the **CALIBRATION** screen (*see Figure 15, repeated below for easy reference*).

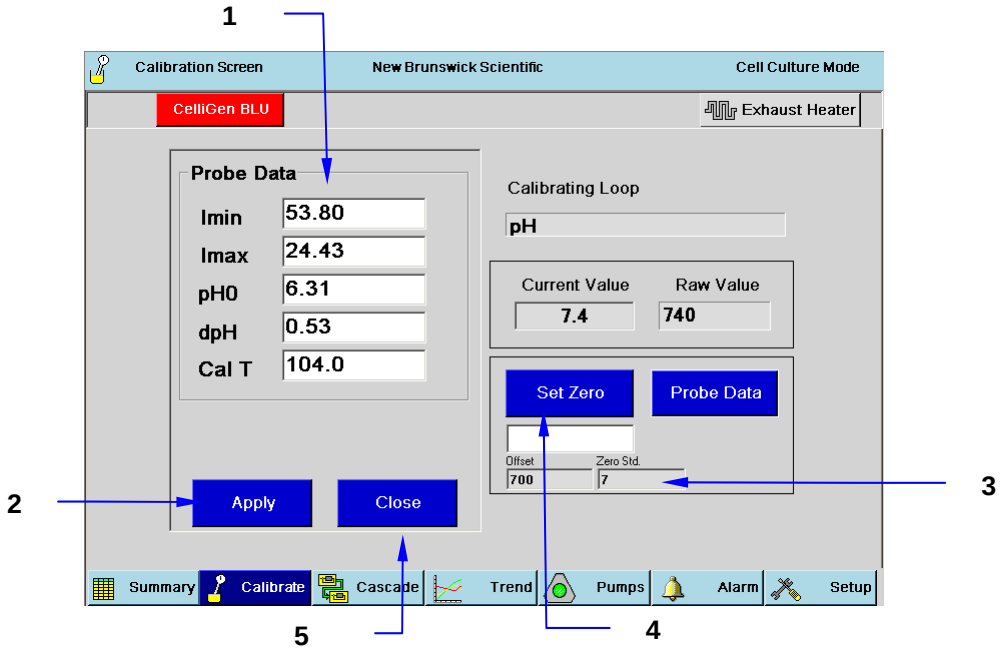
Figure 15: Calibration Screen



1	Step 2a: Press pH here…
2	Step 2b: …and pH appears here (Calibrating Loop box).
3	Step 3: Press the **Probe Data** button to open the PROBE DATA screen (see Figure 15a).

(the instructions continue on the following page)

Figure 15a: Probe Data Screen



1	Step 4: As marked on the label on the bottom of your vessel (and also on the vessel box), enter the Imin, Imax, pH0, dpH and Cal T values in their respective edit boxes here by pressing the box, using the popup keypad to type the number, then pressing the keypad **OK** button.
2	Step 5: When all values have been entered, press the **Apply** button.
3	See Step 7 below.
4	See Step 8 below.
5	See Step 9 below.

6. Take a sample of media (*see Section 16.4 for the sampling procedure*) and measure the pH using the external meter.

7. Press inside the edit box below the **Set Zero** button and, using the popup keypad, enter the value of measured pH from Step 6. Press the **OK** keypad button to save the value.

8. Press the **Set Zero** button.

9. Press the **Close** button.

NOTE:

A Raw Value indication of 9999 means that the values you have entered are incorrect or that the pH probe is either not connected or not functioning properly.

You can expect to see a very slight drift in your pH value over a period of days due to the properties of optical pH sensors. We recommend that you recalibrate the probe every 3-5 days, following the procedure specified above.

7.2 DO Probe Polarization

NOTE:
If the probe has been disconnected from a voltage source (either the unit's O_2 amplifier or a separate polarizing module) for longer than 5 minutes, it will need to be re-polarized.

To re-polarize the DO probe, connect the probe to the operating O_2 amplifier (or polarizing module). Allow six hours for polarization prior to calibrating the probe.

CAUTION!
Never attempt to operate the system if the DO probe is not installed with its cable connected to the control cabinet.

7.3 DO Probe Calibration: Setting Zero

There are two methods to obtain zero for calibrating the DO probe. Review both methods and use the one you prefer:

Method 1:

1. Remove the DO cable from the DO electrode.
2. Go to the **CALIBRATION** screen (*see Figure 15*) and select **DO**.
3. Enter **0** in the **Set Zero** edit box (*see Figure 22 on the following page*), then press **Set Zero**.
4. Reconnect the DO cable to the DO electrode.

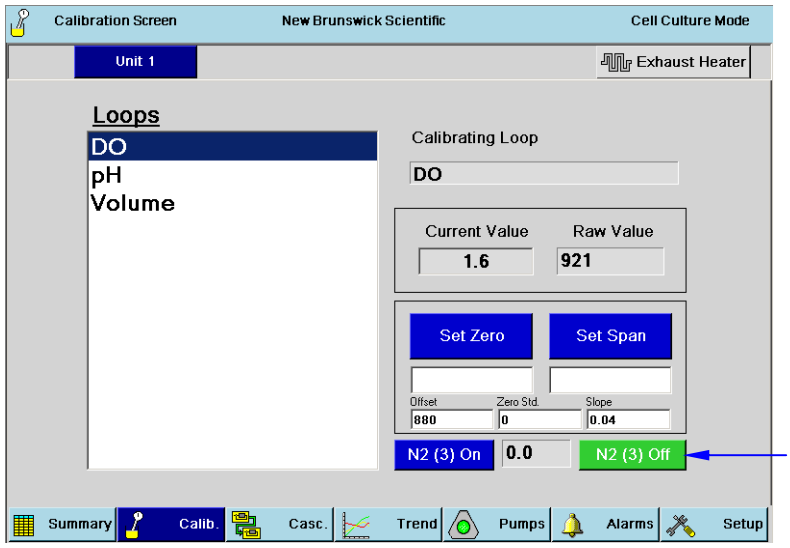
NOTE:
If you use Method 1, make sure the probe is not disconnected for more than two minutes.

Method 2:

NOTE:
Nitrogen is needed for Method 2. There is an N_2 gas inlet on the control cabinet for this purpose; make sure that your nitrogen source is connected to this inlet.

1. Connect the DO cable to the DO electrode and the control cabinet.
2. Go to the **CALIBRATION** screen (*see Figure 15*) and select **DO**.
3. Press the **N2 (3) ON** button (*see Figure 22*). If your system has 3 or 4 TMFCs, however, this button will not be present. In this case, manually turn the N_2 loop on from the **SUMMARY** screen and set it to the maximum setting (depending on vessel size and flow controller).
4. Set Agitation to at least 50 RPM.
5. In approximately 10-30 minutes, the current value reading will stabilize.
6. Press the **Set Zero** edit box (*see Figure 22*), use the touchpad to enter **0**, press the **OK** button, then press the **Set Zero** button.
7. Press **N2 (3) OFF** (or, if in Step 3 you manually turned the N_2 loop on, now manually shut off the nitrogen flow to the vessel).

Figure 22: Calibrating DO



1	The **N2 (3) On** and **N2 (3) Off** buttons will only appear on systems with 1 or 2 TMFCs.

7.4 DO Probe Calibration: Setting Span

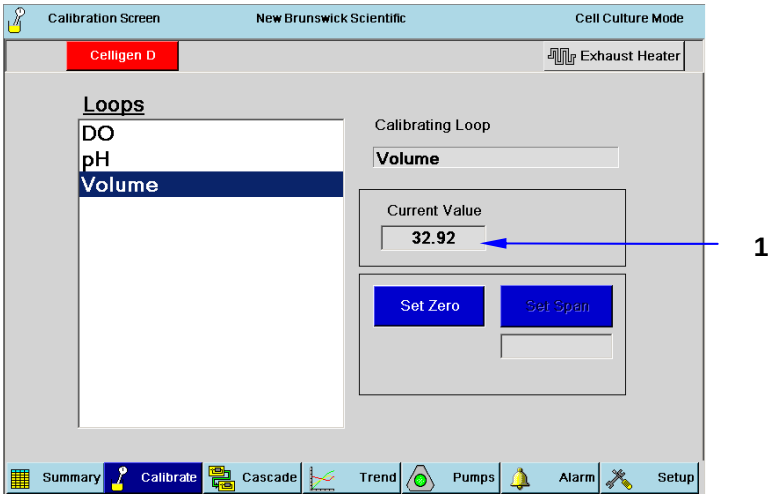
1. In the **AGIT GAUGE** screen, set the **AGIT** speed to **50 RPM**.
2. Set the **AGIT** mode to **AUTO**.
3. Vigorously sparge air into the vessel via the filter on the headplate until the display is stable for approximately 10 minutes (this may take up to 30 minutes total).
4. In the **CALIBRATION** screen, select **DO**.
5. Enter **100** in the **Set Span** edit box (*see Figure 22*), then press the **Set Span** button.

7.5 Scale/Volume Calibration

If you do not have the optional scale, skip this section. The scale is calibrated using a two-point calibration method. First, make sure the scale is properly connected to the control station (*see Figure 2*, optional Scale Connector).

1. Press the **Calib.** button to open the **CALIBRATION** screen, then press **Volume** in the Loops pane.
2. Place a graduated cylinder or Ehrlenmeyer flask on the scale and allow the reading to settle (*see Figure 22a*).

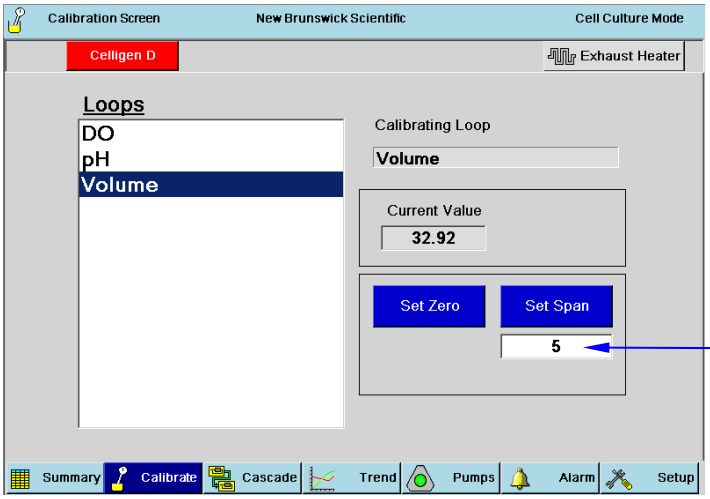
Figure 22a: Setting Zero for Scale/Volume Calibration



1	Allow the Current Value arbitrary reading time to settle.

3. Press the **Set Zero** button.
4. Fill the cylinder or flask to a known quantity of water (e.g., 1.0 liter).
5. Enter the volume in the **Set Span** edit box.

Figure 22b: Setting Span for Scale/Volume Calibration



1	Enter liquid volume here.

6. Place the filled container on the scale, then press the **Set Span** button.
7. Remove the container from the scale and place the complete CelliGen BLU vessel assembly (with motor, heat blanket and exhaust heater but without media) on the scale. Press the **Set Zero** button.

The scale is now calibrated and will measure the weight of liquid added or removed from the vessel.

8 CONNECTING THE VESSEL ASSEMBLY

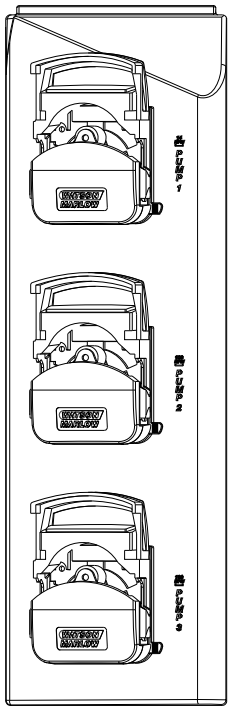
8.1 Connect the Vessel Assembly

1. Position the vessel next to the CelliGen BLU control cabinet.
2. If you have not already done so, carefully position the motor on the bearing housing, on top of the vessel assembly, using the notches to orient it properly.
3. Connect the pH cable to the pH connector on the control cabinet.
4. Connect the DO cable to the DO connector on the control cabinet.

8.2 Load Pump Tubing

The three standard pumps are located on the front of the control cabinet (*see Figure 23):*

Figure 23: Standard Pump Array (pumps open)



Before you insert tubing into the pump channel, verify that the pump is in the **OFF** control mode. Follow these steps to properly load tubing into the pump:

1. Pull the upper pump cover upward to gain access to the interior of the pump.
2. Select the desired tubing size (*see Table 8 in Section 13.3 for reference*) and cut a length sufficient to reach from the inlet source, through the pump, and to the outlet recipient, allowing a few extra inches.
3. Noting that the pumps run in a counter-clockwise direction, place the tubing snugly across the pump channel.
4. Close the upper pump cover by pulling it back down until it clicks into place.
5. Use the pump wheel on the right side (facing the pump) to set the channel to the diameter of your tubing.
6. Press and hold the pump mode **Prime** button or change the pump mode to **ON** at 100% setpoint and ensure that the pump operates smoothly.

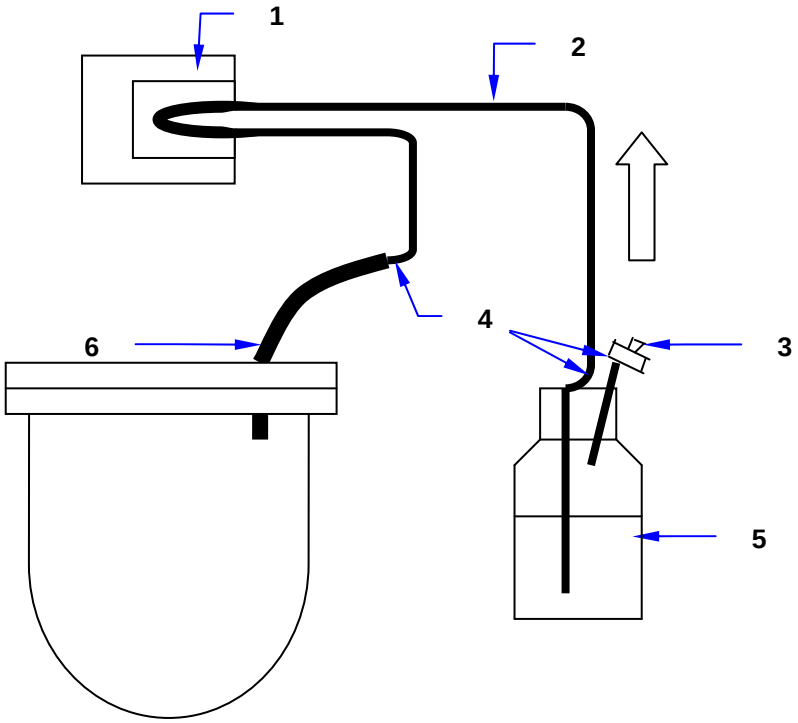
A novel optional feature of these CelliGen BLU pumps is that you can add another identical pump onto the front of one of the pumps in the array (*see Section 0, Appendix B, for details*).

See Section 9.5 for details on pump assignment and Section 13 for details on pump set-up and operation.

8.3 Install Liquid Addition Systems

Figure 24 is a simple depiction of a typical addition system. Depending on the liquids (base, nutrients, media) to be added, your system may be slightly different.

Figure 24: Typical Liquid Addition System



1	Peristaltic pump	4	Plastic ties
2	Tubing	5	Addition bottle
3	Breathing port with sterile filter (0.2μm)	6	Addition port

Be sure to read important CAUTION on the following page.

1. Aseptically install (if applicable) a sterile (0.2μm) filter in one of the two penetrations on the addition bottle cap.
2. Aseptically connect the tubing, securing it with a plastic tie, to the harvest tube in the addition bottle. Clamp it off at the top.
3. If you have not already done so, thread the tubing through the selected feed pump.
4. Connect the tubing, securing it with a plastic tie, to the appropriate addition port on the headplate.
5. Remove the clamp.

CAUTION!

Proper pH control is critically dependent on tubing size, which should be as small as possible. Consult Table 8 for guidance.

8.3.1 Addition Tubing Size

pH can be controlled by automatic additions of liquid base and CO_2. Additions are triggered by the RPC controller, which is constantly comparing current pH value with the pH setpoint and making adjustments as necessary.

The concentration of base, and the inner diameter of the base addition tubing (where it passes through the peristaltic pumps), are critical parameters in the proper operation of a P&I pH control system. If the tubing is too large, excessive doses will be added. The result is that the system will "overcontrol," alternating in close succession between adding base, then CO_2, providing little or no change in pH reading. A user-selected deadband value is an aid to control pH within the user-assigned range: nothing will be added when the pH value falls within the deadband tolerance above or below the setpoint.

5-normal solutions make a good trade-off between moderate addition volume and good control characteristics. The correct tubing diameter varies a little with process, but inside diameters as small as 0.2 mm sometimes eliminate overcontrol while supplying sufficient liquid during high-demand culture phases.

NOTE:

Whatever the tubing ID, the tubing wall thickness must be 1/16-inch (1.6 mm).

NBS suggests that you begin with the supplied tubing, which is correct for most applications. If the system oscillates, reduce the tubing ID where it passes through the pump. Use commonly available step-up/step-down adapters and narrower bore tubing to make the tubing modifications, if required. *Consult Table 8, the flow rate/tubing size chart, for further information.*

8.4 Connect Gases

Ensure that all gas lines (air, oxygen, etc.) are routed to the appropriate ports and secured at both ends with plastic ties.

WARNING!

NEVER OVER-PRESSURIZE A CULTURE VESSEL!

- **Always use eye protection, and exercise caution in the vicinity of culture vessels. If the vessel exhaust becomes blocked, pressure can build up, possibly shattering the vessel and endangering personnel.**
- **Before opening the airflow valve(s), visually confirm that the vessel exhaust is not blocked by kinked tubing, clamps or a wet filter.**
- **After opening the airflow valve(s), verify by feel that air is flowing freely from the exhaust. If not, immediately close the valve(s) or turn off the air/gas supplies.**
- **Never intentionally block the exhaust to raise vessel pressure.**
- **Use the minimum air/gas pressure that will provide adequate airflow for the application. Never exceed the maximum pressure specified in this manual.**

9 GETTING STARTED

9.1 Control Modes

A control mode is the logic by which a controller generates the desired control signal. The operator has a choice of control modes, the most common of which are **ON, OFF**, **AUTO** and **MANUAL**.

In cascaded control, one sensor influences an actuator that is normally associated with a different sensor. The onscreen control mode choice will be the name of the loop chosen to have influence on the actuator.

9.2 Setting P & I Values

P & I values are numbers that determine how the bioreactor responds to changing growth conditions and new setpoints. These are listed in each loop's **GAUGE** screen. You may need to modify P&I values to suit your particular process. To do so, press inside the Proportional & Integral edit boxes, each time entering the desired value using the popup keypad.

NOTE:
If you change P&I values, you can return to the original settings at any time by pressing the Factory Default button (*see Figure 10*).

9.3 Loop Setpoints

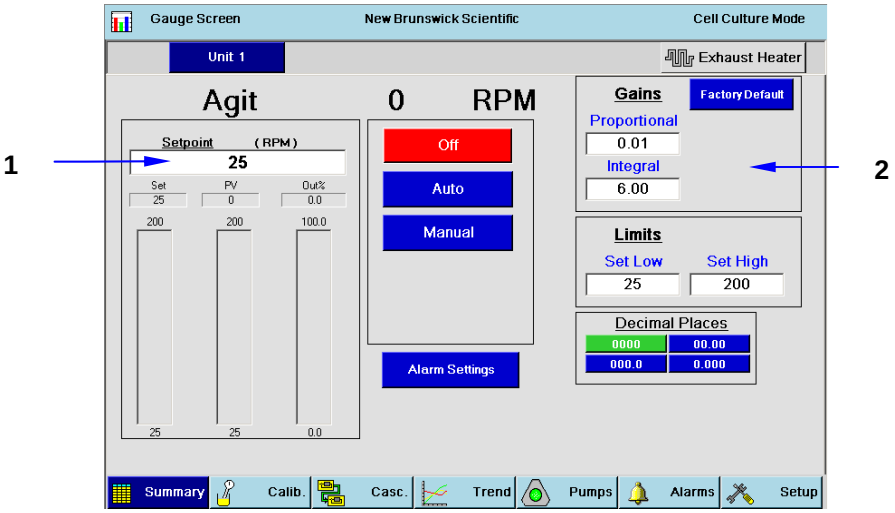
The setpoint is the value you want each loop to attain. When the loop control mode is **AUTO**, the bioreactor will automatically make appropriate adjustments to maintain the value at the setpoint.

9.3.1 Entering Setpoints

To enter a setpoint for any loop, follow these steps:

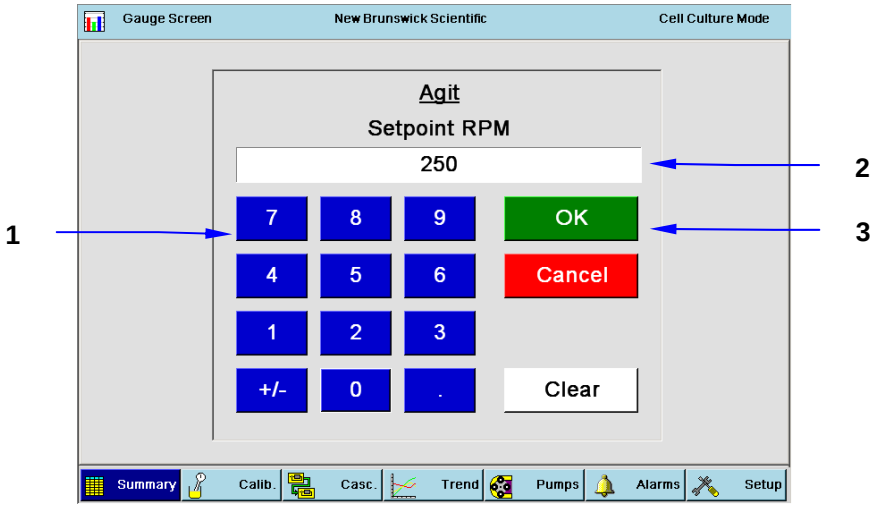
1. Touch either the **LoopName** box or the **Setpoint** box for the desired loop on the **SUMMARY** screen. In this example, we have selected AGIT.
2. The loop **GAUGE** screen opens (*see Figure 10, repeated on the following page for easy reference*):

Figure 10: Sample GAUGE Screen



1	Step 3: Press inside the Setpoint box to open the touchpad (see Figure 25 to continue)
2	P&I Values: Adjusting these values will determine how your system responds to changes in your culture. See Section 22.5 for more information.

Figure 25: Setpoint Touchpad



1	Step 4: Use the touchpad number keys to enter the desired Setpoint. Use the white **Clear** button at any time before Step 5 to empty the Setpoint edit box.
2	Note that Agitation cannot be set higher than 200 RPM; the system will default to 200 if you try to input a higher number.
3	Step 5: Press the **OK** button to save the setpoint and to return to the GAUGE screen, or press the **Cancel** button to return without saving the setpoint.

9.3.2 Modifying Setpoints

This process is the same as entering setpoints. *See Section 9.3.1 above.*

9.4 Cascade System

Cascading brings several systems together to work jointly for the achievement of your goal. An example of how this works would be controlling pH by using a cascade from a pump: as the pH alters from the setpoint, the program would cause the pump to turn on and off to compensate.

See Section 10 for details about setting cascades.

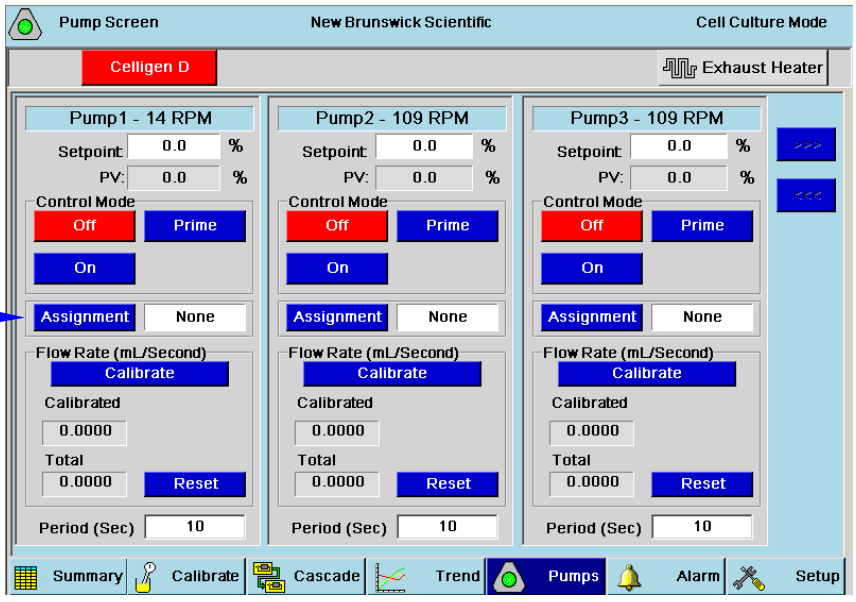
9.5 Pump Assignment

The user has the ability to assign each pump present in the system.

To assign a pump:

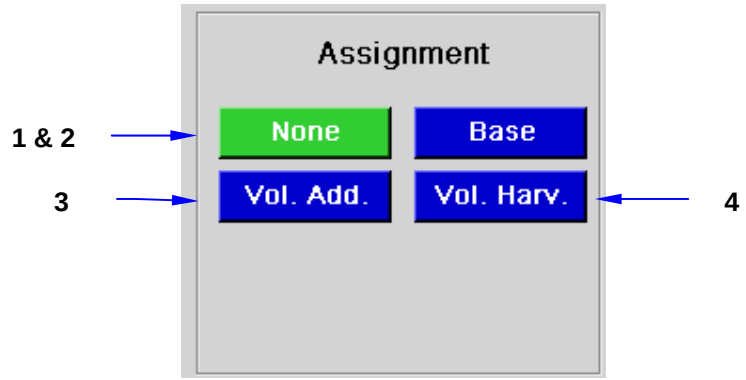
1. From any screen, press the **PUMPS** button at the bottom to open the **PUMPS GAUGE** screen (*see Figure 18*):

Figure 18: Pumps Screen



1	Step 2: Press the Pump1 **Assignment** button to show the Pump Assignment pane (see Figure 26 to continue).

Figure 26: Pump Assignment Pane



1	Step 3: Press the button that corresponds to your choice of assignment for Pump1.
2	Step 4: Repeat Steps 2 & 3 for the other pump(s) to be assigned.
3	Vol. Add. = Volume Addition (see Section 9.7.1)
4	Vol. Harv. = Volume Harvest (see Section 9.7.2)

5. Press the **Summary** screen access button to save the pump assignment(s) and to return to the **SUMMARY** screen.

9.6 Pump Calibration

Pump flow rates are provided in Table 8 (*Section 13.3*). However, more accurate flow rates through the various lines may be established by pre-calibrating the pumps, using the **PUMPS** screen (*for details, see Section 13.3*). This screen controls all pump parameters for the three standard fixed speed pumps supplied with the control cabinet and for any additional pumps added through the available analog input and output connections.

Using the **PUMPS** screen, you can view total pump flow rate in ml/second, set the pump's cycle time, and assign each pump to one of four functions (None, Base, Vol Add and Vol Harv; *these last two are explained in Section 9.7*).

NOTE:
To assure the most accurate flow rate, calibrate the pump (*see Section 13.3*) each time you change tubing.

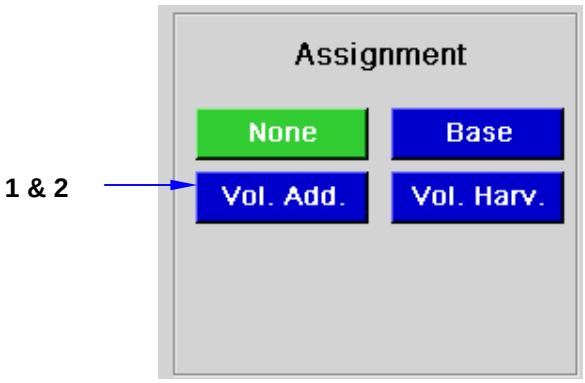
9.7 Using Optional Scales to Program Pumps

9.7.1 Setting a Feed Pump to Add Liquid

A feed pump can be set to add liquid whenever the optional load cell/scale informs the pump that an addition is needed to maintain level.

1. Open the **PUMPS** screen.
2. Select the feed pump you wish to pump liquid from your addition system (*see Section 8.3*) into the vessel, and press that pump's **ASSIGNMENT** button to open the **PUMP ASSIGNMENT** pane (*see Figure 26*, repeated here for easy reference).

Figure 26: Pump Assignment Pane



1	Step 3: Press the **Vol. Add.** button.
2	Step 4: Press the **Summary** screen access button to save the pump assignment and to return to the SUMMARY screen.

9.7.2 Setting a Pump to Harvest Vessel Contents

A pump can be set to harvest liquid when the scale informs the pump that the harvest setpoint has been reached.

1. Open the **PUMPS** screen.
2. Select the pump you wish to harvest contents from your vessel (after aseptically setting up your harvest system), and press that pump's **Assignment** button to open the **PUMP ASSIGNMENT** pane (*see Figure 26* above).
3. Press the **Vol. Harv.** button, then press **Summary** to save the pump assignment and to return to the **SUMMARY** screen.

10 CASCADE CONTROL

Cascades are control schemes in which the Output % of one process control loop influences the setpoint of one or more other loops. In other words, it uses feedback from one parameter to influence others. In New Brunswick Scientific's CelliGen BLU bioreactors, the output % value is mathematically determined by evaluating the error between measured present values and desired setpoints, and integrating these values into a PID-based control algorithm.

The CelliGen BLU's RPC controller allows cascading from any loop to as many as five other loops. DO and pH are the most commonly *cascaded-from* loops; oxygen and nitrogen commonly receive the cascade from DO, and CO_2 and Base pump usually receive the cascade from pH, altering their respective setpoints to correct errors in DO.

When more than one loop is configured as the recipient of a cascaded loop, they may respond in parallel, at the same time, or in series, one after the other, depending on how the cascade has been set up. Cascades set up to run in series generally give more predictable control responses. Sometimes a small region of overlap, where two loop setpoints vary simultaneously, is used to smooth the transition from one loop to another.

To enable cascades, gas loops must be in **Manual/Cascade** mode, which can be selected from the **GAUGE** screens, and all other loops involved in the cascades must be set to **Auto** or, in the case of pumps, set to **ON**.

- When systems are configured with multiple TMFCs and **Manual/Cascade** mode is selected, the controller automatically populates default cascade values for the gases being used, but it is important to make sure all loops involved in the cascades are set to **Manual/Cascade** mode. Controllers configured with 0 or 1 TMFC are not automatically populated with cascade values when **Manual/Cascade** mode is selected; they must be entered manually.

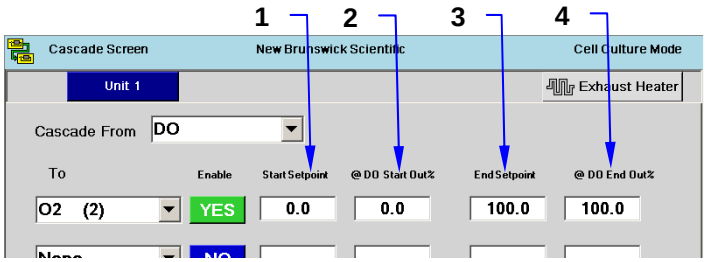
- Each cascade must be set to **Enabled** (*see Figure 16 in Section 10.1*).

The default cascade values can be overwritten, and customer values can be entered using the procedures outlined in this section.

10.1 Creating a Cascade

Figure 16a below shows the headers from the **CASCADE** screen (set to "Cascade From DO"), with an explanation of the settings those headers represent:

Figure 16a: Cascade Screen



1	**Start Setpoint** is the loop value the user defines for the system to be at when initial **DO Start Out%** is reached. Typically this value will be close to the normal operating setpoint.
2	**@DO Start Out%** represents the DO output % value where the user wants the cascade to begin. When this output % is reached, the setpoint of the Cascade To loop will change to the value entered as Start Setpoint. The current DO output % can be found on the **SUMMARY** screen at the intersection of the Output% column and the DO loop row. This value is calculated by using the integrated PI values. It is essentially a mathematical calculation of setpoint "error" from PV (current process value), "error" meaning any readings that are above or below the programmed setpoint. As the "error" discrepancy increases, or as the duration of such a discrepancy remaining static increases, the Output% also increases.
3	**End Setpoint** is the loop value the user defines as the maximum allowable value when the **DO End Out%** is reached. Typically this value will also be the same as the system's maximum allowable setpoint for the loop.
4	**@DO End Out%** represents the DO output % where the user wants the cascade to stop. This value can be set to any integer from 0 to 100% as long as it is greater than the Start Out%. The greater it is than the Start Out%, the smoother the increase in setpoints.

NOTE:

It is important to remember that cascades are based on the loop (whether DO or pH) Out% value posted in the SUMMARY screen. These numbers are the basis for all cascades involving that loop. See the examples below for more explanation.

It can be a very beneficial exercise to watch how the values on the **SUMMARY** screen change to reflect differences between the present value (PV), the setpoint, and the DO output percentage (Out%).

When the PV is greater than the setpoint, the system will be generating a negative Out% because the controller senses a need to decrease DO:

LoopName	PV	Setpoint	Out%	Control Mode	Units	Casc.
DO	74.8	35.0	-38.6	Auto	%DO	Source

When the PV is less than the setpoint, the system will be generating a positive Out%, because the controller senses a need to increase DO:

LoopName	PV	Setpoint	Out%	Control Mode	Units	Casc.
DO	25.2	35.0	51.6	Auto	%DO	Source

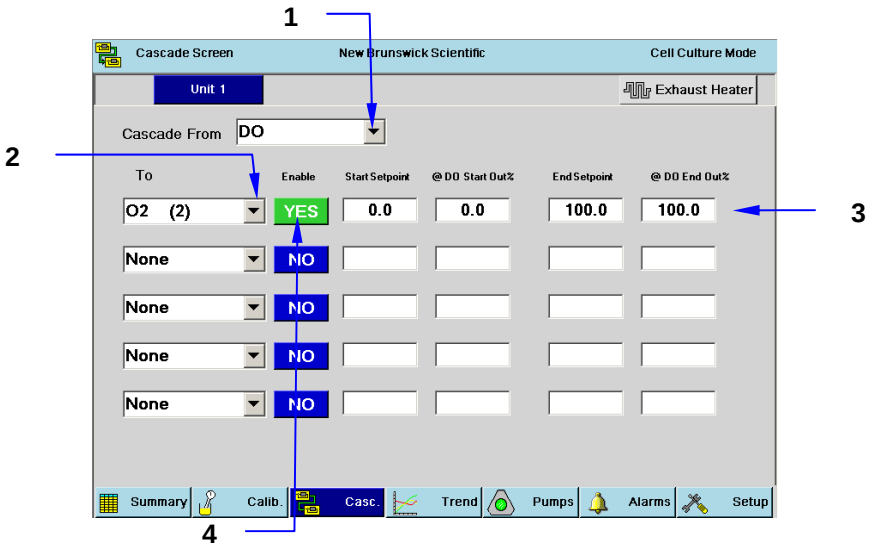
When the PV equals the setpoint, the Out% should be approximately 0, as the controller senses no need to make adjustments to DO:

LoopName	PV	Setpoint	Out%	Control Mode	Units	Casc.
DO	35.2	35.0	0.0	Auto	%DO	Source

To create a cascade:

1. Press the **CASCADE** button to open the **CASCADE** screen (*see Figure 16*, repeated below for easy reference):

Figure 16: Cascade Screen



1	Step 2: Use this ▼ dropdown menu to select the "Cascade From" loop.
2	Step 3: Use the first ▼ dropdown menu to select the first "Cascade To" loop.
3	Step 4: Set the **Start Setpoint**, **@DO Start Output%**, **End Setpoint** and **@DO End Output%** values one by one by pressing the edit box, entering the desired value on the touchpad and pressing the **OK** button.
4	Step 5: Press the **YES** button to enable this cascade.

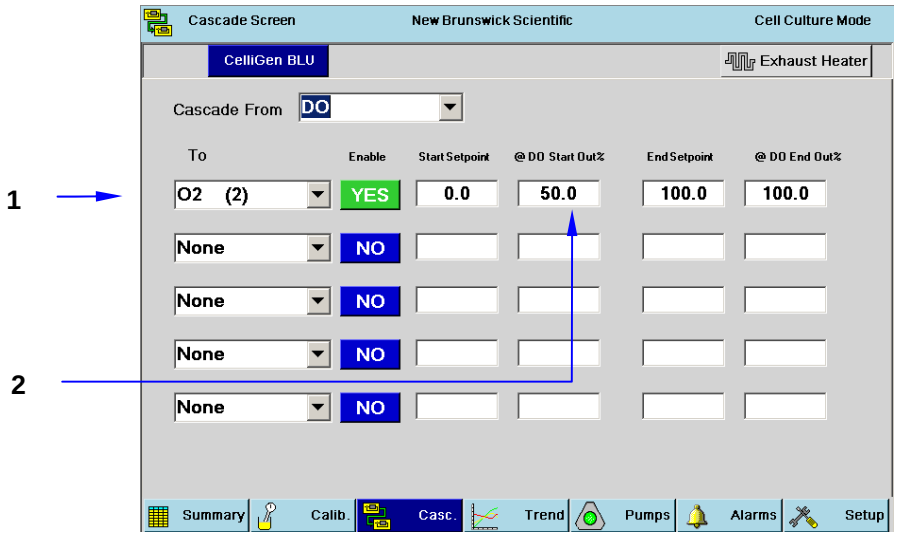
10.2 Controlling DO by Cascade

Example: Cascading DO to Air and Oxygen.

In this example (*see Figure 27*), **DO** is cascaded to **O2 (2)**.

NOTE:
Figure 27 is an example of a DO cascade for a system configured with 0 or 1 TMFC. Gases are represented in ranges between 0-100%.

Figure 27: Sample DO Cascade



1	O2 **Start** & **End Setpoint**s are set to increase between 0-100%.
2	This column indicates DO Output % at the beginning of the cascade.

Assuming that DO output ranges from -100% to 100%, a cascade setup that is enabled as explained below will cause the air and oxygen setpoints to vary with DO output. If the % air alone cannot maintain the DO setpoint, O2 % will increase to add oxygen or a combination of air + oxygen.

In the cascade shown above, once the DO Output% increases to 50% (the PV is below the SP), the oxygen loop begins to increase from its staring setpoint of 0% to an ending setpoint of 100%. As the O_2 increases, the Air will decrease by the inverse percentage. The total Gas % will remain 100%.

Alternately, N2 can be used in a cascade loop to drive the DO down. Using this method, when the DO output% decreases below zero, nitrogen is directed to lower the DO.

Cascades like this can also be set up for pH where the PV and SP differences cause the percentage or flow rate of CO_2 or a pump to react in response.

NOTE:

- **Regardless of DO (*cascaded-from*) output, the setpoint of any *cascade-to* loop will not go below its own Minimum Setpoint value.**
- **Minimum Output% corresponds to the minimum value that will produce the minimum setpoint; lower outputs will not affect setpoint.**
- **Regardless of DO output, the setpoint of any *cascade-to* loop will not rise above its own maximum setpoint.**

11 GAS MIXING FOR PH & DO CONTROL

Due to the sensitivity of the cells, pH and DO in cell culture processes are typically managed through gas mixing. The addition of CO_2 into the media through the sparge line is usually used to maintain pH. The introduction of this gas causes the pH of the culture to decrease due to a slow accumulation of bicarbonate in the solution. On the other hand, decreasing the CO_2 sparge rate allows the pH to drift up, either due to the natural alkaline drift of the media or because of the accumulation of alkaline waste products in the solution.

DO is maintained in solution by introducing Air, O_2 and sometimes N_2 in various combinations or at different flowrates. The controller calculates which and how much of either of these gases is required to maintain the user-selected DO setpoint.

Your system can come equipped with 0, 1, 3 or 4 thermal mass flow controllers (TMFC) and up to one overlay with or without a TMFC. If you do not know how your system is configured, this information can be found on the **SETUP** screen, under the Controller Set-Up tab. The number of TMFCs and their flow rates are factory-set and cannot be adjusted.

Your CelliGen BLU has the ability to adjust pH and DO by increasing or decreasing the individual gas concentrations within the vessel. There is a wide variety of system configurations possible with respect to gas control. This section will help explain these choices and will guide you through their operation.

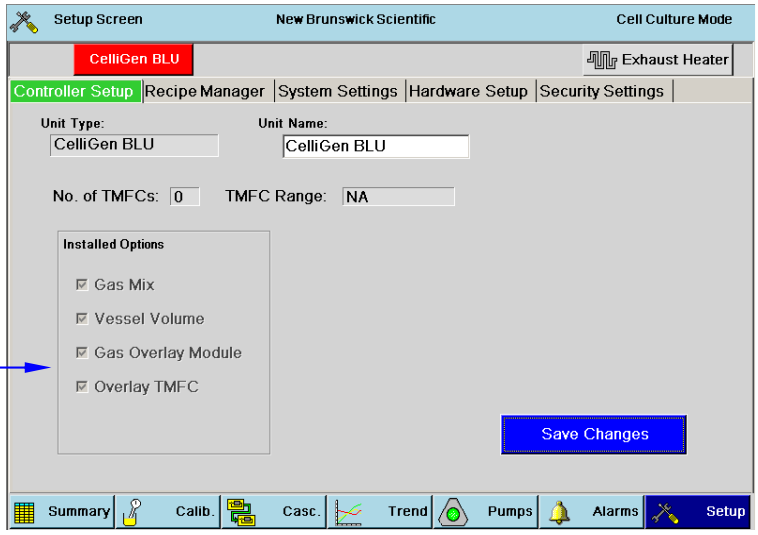
Depending on how your system is configured, you may find that the high limit for CO_2 is below the high limit of its associated flow controller. This limit is imposed to prevent oxygen starvation while adjusting pH with CO_2.

Your system is capable of operating in either 3-gas or 4-gas control mode. 3-gas uses air and O_2 to regulate DO and CO_2 to regulate pH; 3-gas mode does not include N_2. 4-gas mode uses air and O_2 as well as N_2 for the control of DO and CO_2 to control pH.

11.1 Gas Control with 0 TMFC

If your system is configured with 0 TMFC, gas mixing is controlled through a bank of 4 solenoids and a rotameter is used to manually control the flow. The software will still control how the solenoids open and close and you will still be able to select a control mode from the gauge screen, but the overall flow will be regulated manually by the rotameter (*see Figures 28, 29a, 29b, 29c & 29d on the next pages*).

Figure 28: Controller Setup Screen (0 TMFC)



1	See Section 11.5 for details on the Gas Overlay.

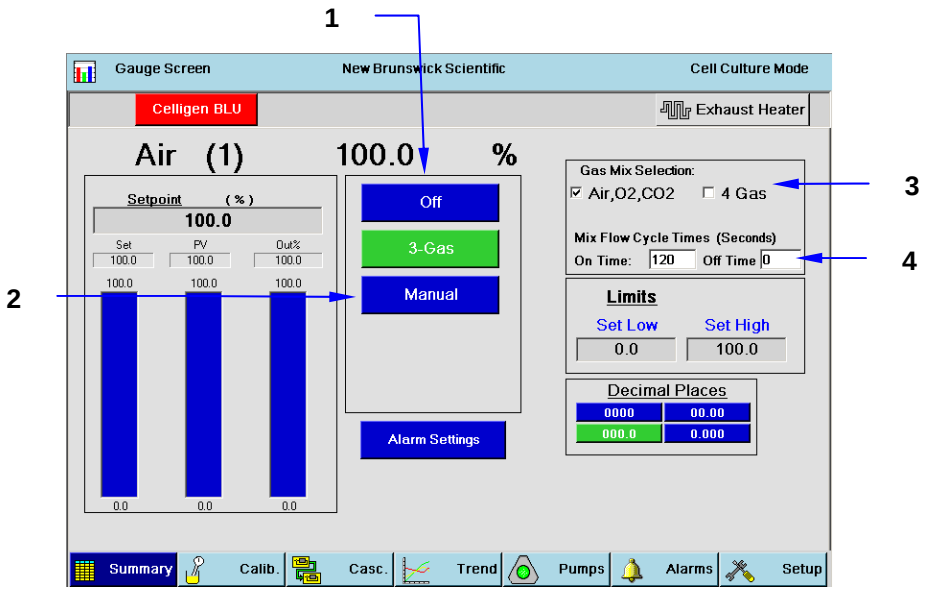
The system will automatically generate three or four gas process loops on the **SUMMARY** screen. These loops are labeled: Air (1), O2 (2), N2 (3) and CO2 (4). Their numbers 1-4 correspond to the gas connections on the cabinet (*see Figure 2*). When you open the gauge screen for any of these gas loops you will find a series of buttons for flow control.

The gauge screen for each of the gases will have the option for 3- or 4-gas mixing but no cascade feature to the GasFlo loop. Total gas flow is regulated manually by means of an external rotameter. In addition, if 3-Gas (Air, O_2 and CO_2) or 4-Gas (Air, O_2, CO_2 and N_2) mode is selected, the controller will automatically maintain gas composition percentages based on pH and DO.

The Manual gas mix control button allows you to manually adjust the gas mixture (*see Figures 29c & 29d*).

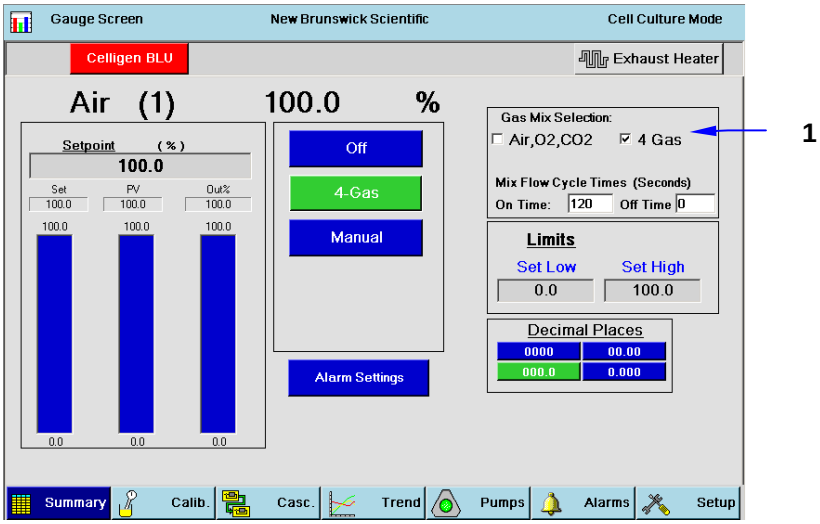
Whatever mixture you set, Air always makes up any difference to complete the 100% total gas.

Figure 29a: Air (1) Gauge with 0 TMFC & 3-Gas Control



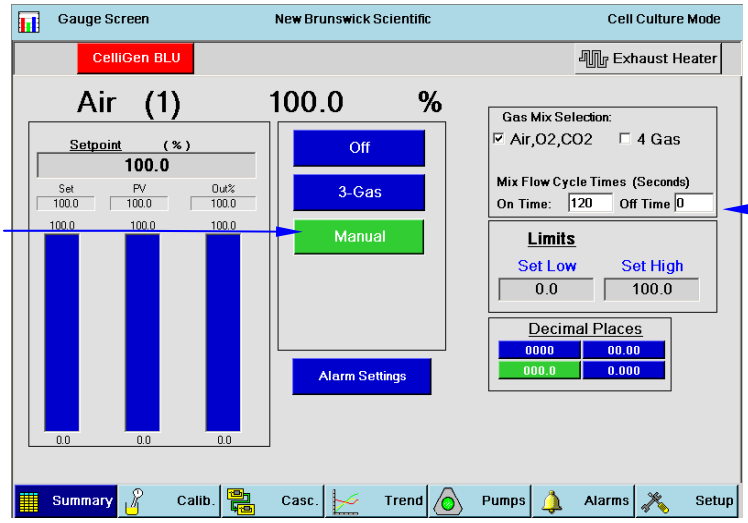
1	Gas Mix Control buttons
2	Press the **Manual** button to control gas mix manually.
3	If you select a 4-gas mix here, the **3-Gas** Gas Mix Control button will change to **4-Gas**, allowing you the option of switching to automatic 4-Gas control.
4	Set Mix Flow Cycle Times (typically used in microcarrier applications) by indicating here the number of seconds each gas should cycle On and Off.

Figure 29b: Air (1) Gauge with 0 TMFC and 4-Gas Control



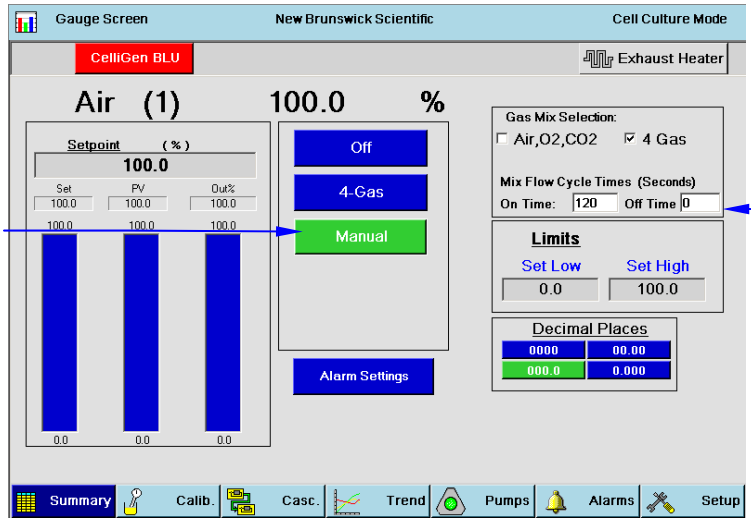
1	If you select **Air, O2, CO2** here, the **4-Gas** Gas Mix Control button will change to **3-Gas**, allowing you the option of switching to automatic 3-Gas control.

Figure 29c: Air (1) Gauge with 0 TMFC & Manual 3-Gas Control



1	Press the **Manual** button to control gas mix manually. You must now control the Air, O_2 & CO_2 percentages manually either by the use of cascades or by entering gas percentage values in each gas loop gauge screen.
2	Set Mix Flow Cycle Times (typically used in microcarrier applications) by indicating here the number of seconds this gas should cycle On and Off.

Figure 29d: Air (1) Gauge with 0 TMFC & Manual 4-Gas Control



1	Press the **Manual** button to control gas mix manually. You must now control the 4 Gas (Air, O_2, CO_2 & N_2) percentages manually either by the use of cascades or by entering gas percentage values in each gas loop gauge screen.
2	Set Mix Flow Cycle Times (typically used in microcarrier applications) by indicating here the number of seconds this gas should cycle On and Off.

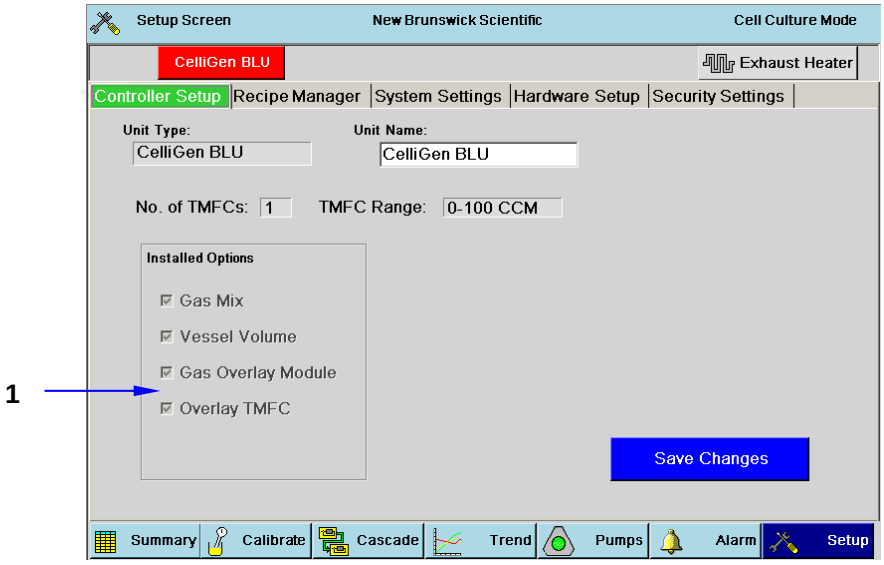
The flexibility of gas control through these gauge screens makes it easy for you to change gas mixes during the various phases of your cell culture run. As you change mixes, if you change the number of gases entering the vessel, your choices will be reflected in the process loops that appear in the **SUMMARY** screen.

Systems with 0 or 1 TMFC will still allow you to set manual control of the gas mix and gas flow. It is important to remember than when the system is set to **Manual**, it will not automatically change the mix based on pH or DO, and that whatever mixture you set, Air always makes up any difference to complete the 100% total gas.

11.2 Gas Control with 1 TMFC

If your system is configured with 1 TMFC, gas mixing is controlled through a bank of 4 solenoids and the gas flow is controlled automatically by means of the TMFC GasFlo process loop. The software controls how the solenoids open and close and you will still be able to select a control mode from the gauge screen, but the overall flow will now be regulated by setting a value for the TMFC.

Figure 30: Controller Setup Screen (1 TMFC)

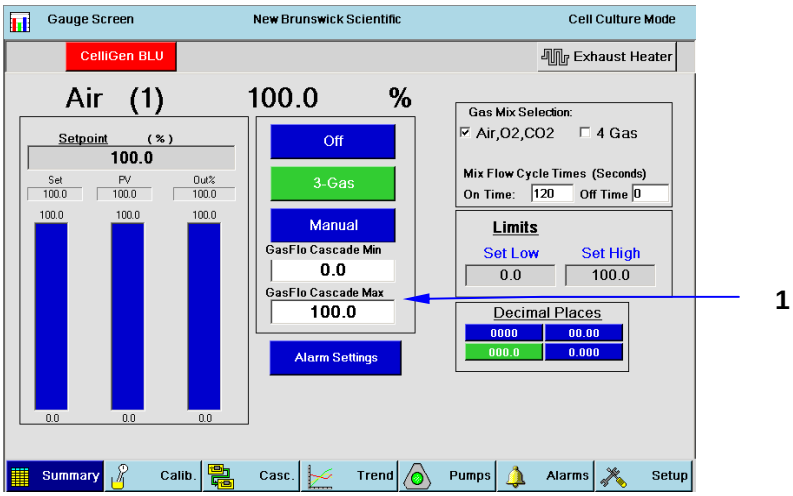


1	See Section 11.5 for details on the Gas Overlay.

The gauge screens for each of the gases will have the option for 3- or 4-gas mixing and, in 3-Gas mode, will also have the GasFlo cascade feature. Gas mixing selections allow the system to automatically regulate gas composition to maintain pH and DO setpoints. To activate the gas control, it is important to set one of the gas gauge screens to 3 Gas, 4 Gas or Manual (*see Figures 31a, 31b, 31c & 31d*).

The GasFlo cascade feature, which is automatically enabled with 3-gas mixing, allows you to define the high and low settings for a GasFlo cascade directly from a gas gauge screen (*see Figure 31b*). The cascade will regulate gas flow from minimum to maximum based on the system's pH and DO demand. If the system has no demand for increasing DO or adjusting pH, the GasFlo will go to the GasFlo Cascade Min setting. As soon as the system calculates a demand, GasFlo will begin to increase.

Figure 31a: Air (1) Gauge Screen with 1 TMFC & 3-Gas Control



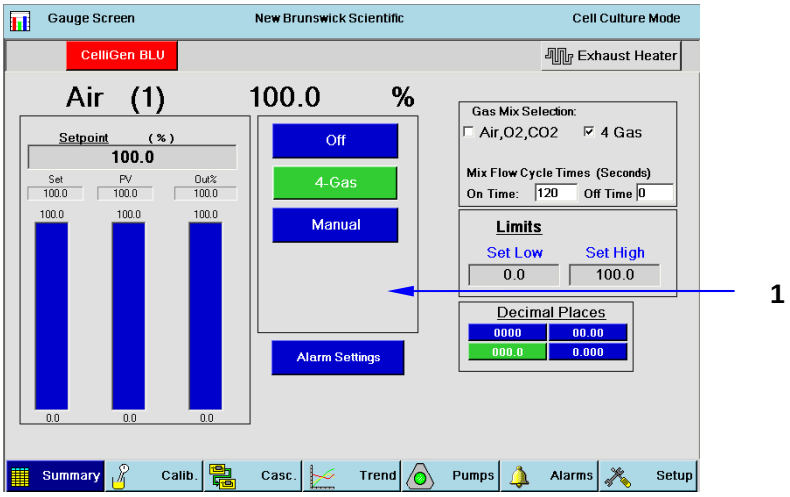
1	GasFlo Cascade minimum & maximum settings

In 3-gas mode, the controller automatically adjusts the gas (Air, O_2 & CO_2) percentages to control pH and DO. Total gas flow from the TMFC can be directly linked to a cascade from this screen using the default GasFlo Cascade values (*see item 1 in Figure 31a*) or setting your own. This allows you to minimize the amount of gas going into the vessel when DO correction is needed; it also limits the total gas flow to avoid foaming. In this control mode, flow will automatically adjust based on both pH and DO demands.

In 3-gas mode, we suggest that you always use an overlay if possible to help maintain your setpoints.

In 4-Gas control mode (*see Figure 31b*), the controller automatically adjusts the gas (Air, O_2, CO_2 and N_2) percentages to control pH and DO. Total gas flow is digitally controlled and can either remain constant or be manually linked to a cascade in the individual gas gauge screens. GasFlo cascade is not automatically available in 4-Gas mode.

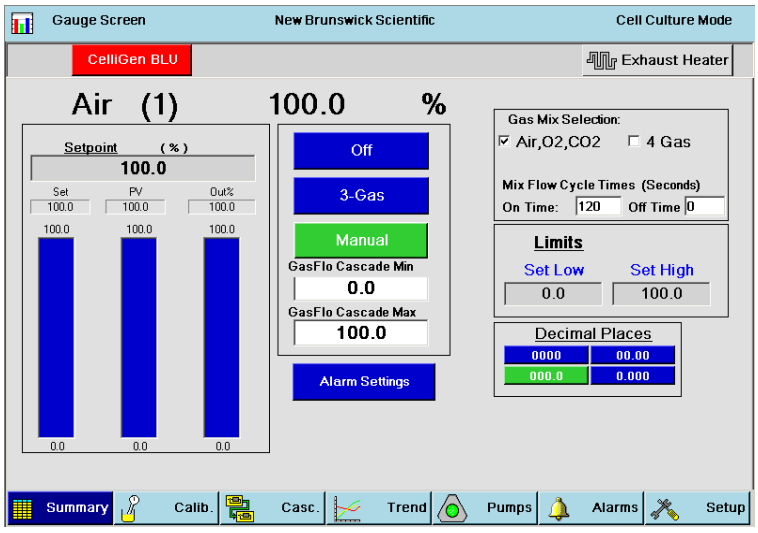
Figure 31b: Air (1) Gauge Screen with 1 TMFC & 4-Gas Control



1	GasFlo Cascade is not automatically available in 4-Gas mode.

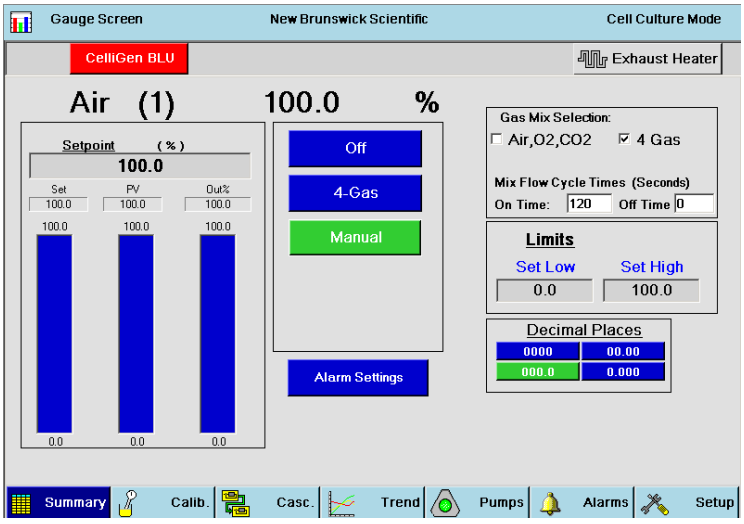
In Manual 3-Gas control mode, when you press the **Manual** control mode button and select **Air, O_2, CO_2** in the Gas Mix Selection pane of any gas gauge screen, you must also manually enter setpoints for each gas in its gauge screen or create a user-defined cascade to control the gas mix. A GasFlo cascade can be set from this screen, entering values in the **GasFlo Cascade Min** and **Max** edit boxes to automatically increase and decrease the overall gas flow based on pH and DO demand.

Figure 31c: Air (1) Gauge Screen with 1 TMFC & Manual 3-Gas Control



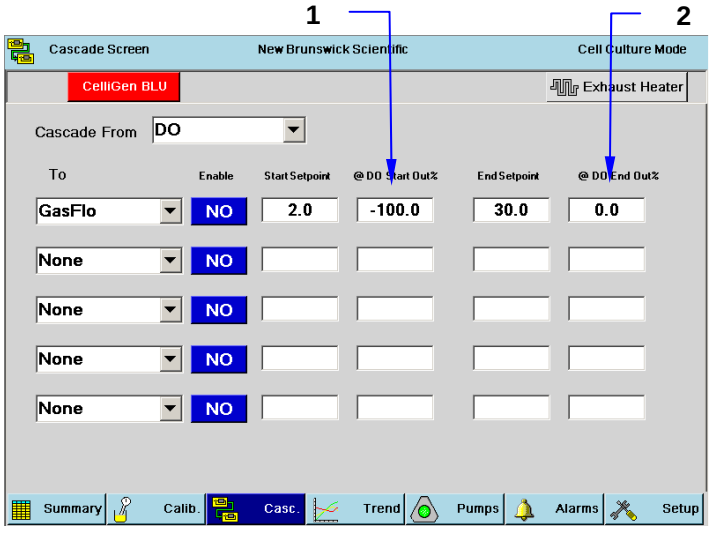
In Manual 4-Gas control mode, when you press the **Manual** control mode button and select **4-Gas** in the Gas Mix Selection pane of any gas gauge screen, you must also manually enter setpoints for each gas in its gauge screen or create a user-defined cascade to control the gas mix.

Figure 31d: Air (1) Gauge Screen with 1 TMFC & Manual 4-Gas Control



To fine tune your cascade settings, you can adjust the Output% and Setpoints in the Cascade screen (*see Figure 31e for a sample Cascade screen and Section 10 for details on Cascades*).

Figure 31e: Sample Cascade Screen with 3- or 4-Gas Mix



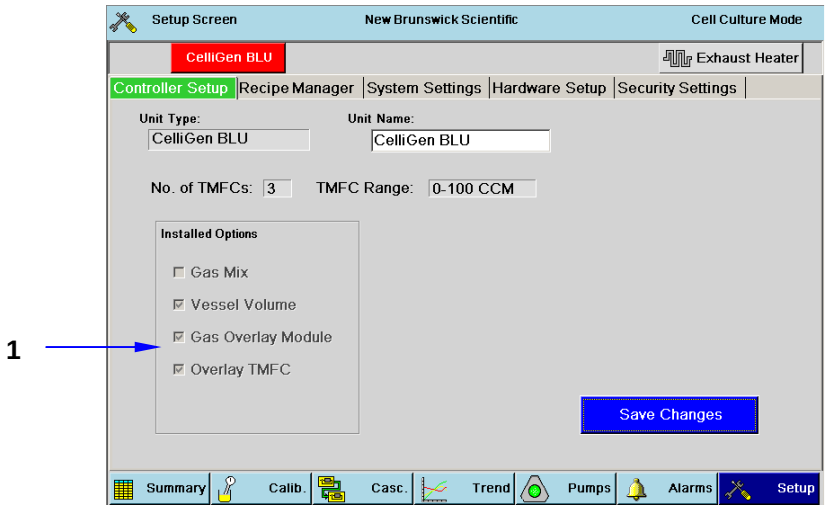
1	DO starting Output %	2	DO ending Output %

NOTE:
When your system is equipped with 1 TMFC and 4 solenoids, you may see some fluctuation in the GasFlo loop PV (process variable) as the solenoids pulse between gases. This is completely normal.

11.3 Gas Control with 3 TMFCs

If your system is equipped with three TMFCs, there are no solenoids and you have access to **Manual/Cascade** or **3-Gas/Auto** control modes in the gas gauge screens (*see Figures 32a & 32c*).

Figure 32: Controller Setup Screen (3 TMFCs)

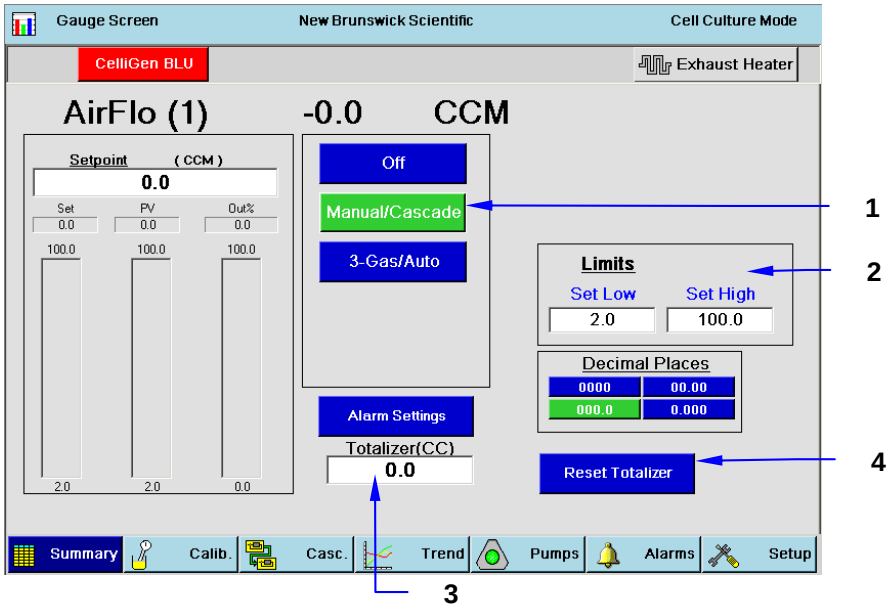


1	See Section 11.5 for details on the Gas Overlay.

The gas process loops you will find in the **SUMMARY** screen are labeled AirFlo (1), O2Flo (2) or CO2Flo (3). Their numbers 1-3 correspond to the gas connections on the cabinet (*see Figure 2*).

With three TMFCs, when you set any gas gauge to **Manual/Cascade** (*see Figure 32a on the next page*), Air, O_2 and CO_2 setpoints can either be manually entered in their individual gas gauge screens or they can be controlled by cascades (*see Figure 32b on the next page*).

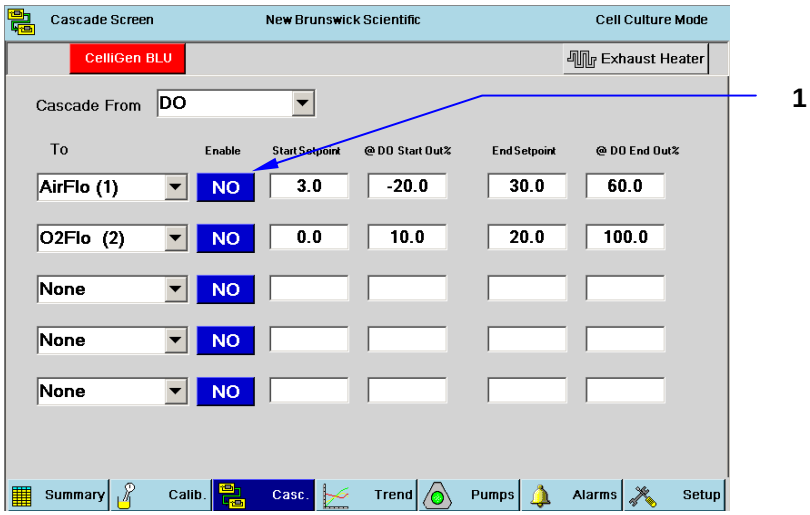
Figure 32a: AirFlo (1) Gauge with 3 TMFCs in Manual/Cascade Mode



1	**Manual/Cascade** mode has been selected; the button is green.
2	**NOTE:** Gas flow limits are 2-100 CCM (SLPM), not percentages.
3	The Totalizer feature tracks and displays the accumulated flow for the associated loop; here it measures accumulated air flow.
4	Use the **Reset Totalizer** button at any time to return to 0.0 and begin counting again.

NOTE:
To reduce the risk of clogging the microsparge, gas flow should not be set below +2 CCM.

Figure 32b: Sample Cascade Screen with 3 TMFCs

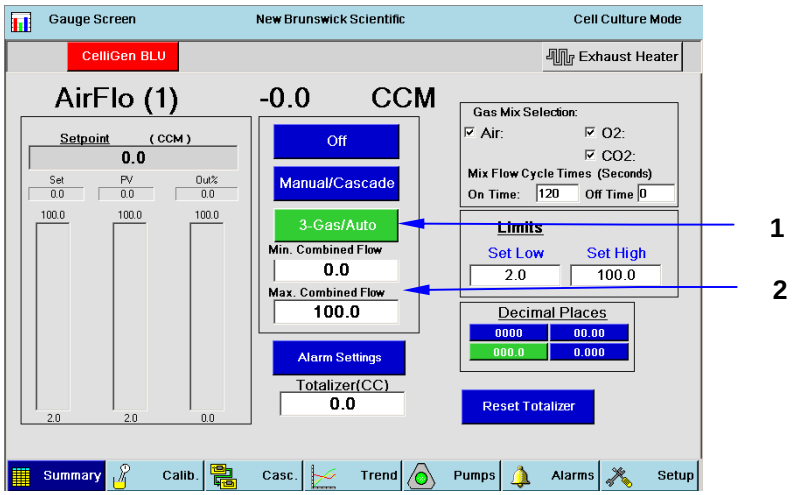


1	Press each loop's **Enable** button to activate automatic cascade control.

Cascade values are automatically controlled but first they need to be activated by selecting each loop's **Enable** button in the **CASCADE** screen (*see Figure 32b*). Default values are provided as starting points; they may need to be modified for the optimization of individual processes.

When you set the system to the **3-Gas/Auto** control mode, the controller automatically adjusts the flow ratio of Air, O_2 and CO_2 based on the demands of pH and DO. The **Minimum** and **Maximum Combined Flow** fields allow limits to be set to the total gas flow so you can prevent too much gas being injected while also maintaining a total positive flow.

Figure 32c: AirFlo (1) Gauge with 3 TMFCs in 3-Gas/Auto Mode



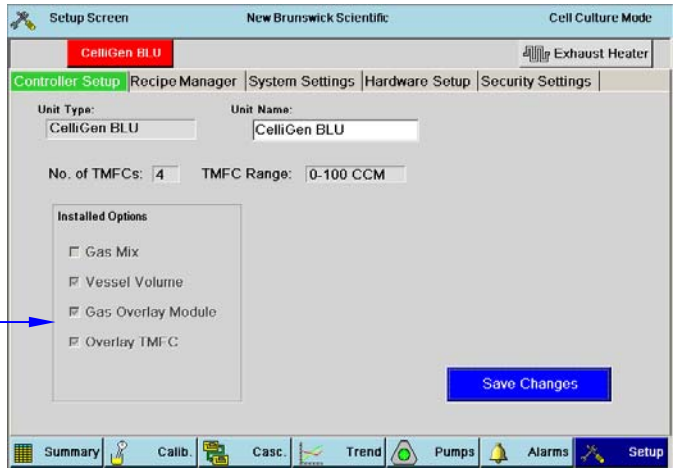
1	**3-Gas/Auto** mode has been selected; the button is green.
2	**NOTE** that these fields are not for GasFlo Cascade; they are for Minimum and Maximum Combined Flow settings, as explained in the text above this figure.

11.4 Gas Control with 4 TMFCs

If your system is equipped with four TMFCs, you have access to **Manual/Cascade**, **4-Gas/Auto** and **3-Gas/Auto** gas mix control in the gas gauge screens (*see Figures 33a, 33c & 33d*).

With four TMFCs, when you set any gas gauge to **Manual/Cascade** (*see Figure 33a on the next page*), Air, O_2, CO_2 and N_2 setpoints can either be manually entered in their individual gas gauge screens or they can be controlled by cascades (*see Figure 33b*).

Figure 33: Controller Setup Screen (4 TMFCs)



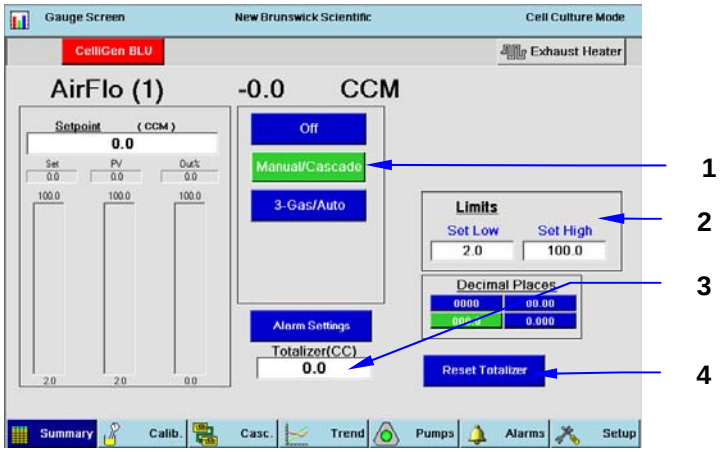
1	See Section 11.5 for details on the Gas Overlay.

The gas process loops you will find in the **SUMMARY** screen are labeled AirFlo (1), O2Flo (2), N2Flo (3) and CO2Flo (4). Their numbers 1-4 correspond to the gas connections on the cabinet (*see Figure 2*).

NOTE:

Any loop name ending in Flo represents a certain gas (e.g., AirFlo, O2Flo, etc.) with a dedicated TMFC. If the gas loop name does not end with "Flo", it represents the presence of a gas solenoid valve rather than a TMFC.

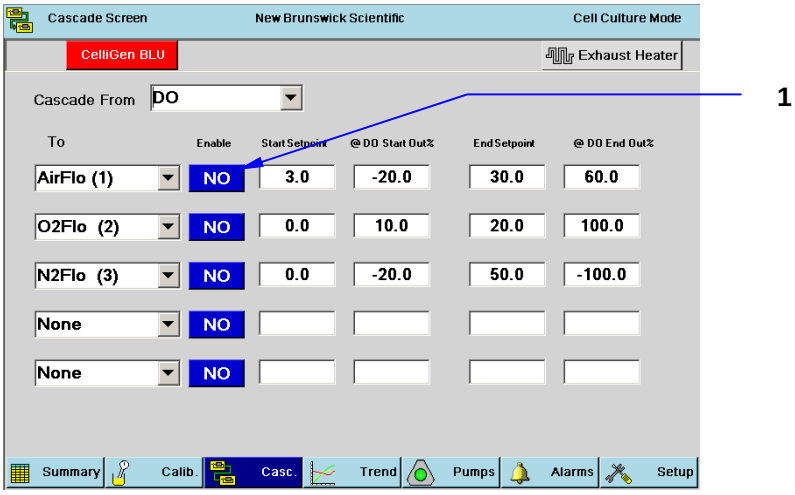
Figure 33a: AirFlo (1) Gauge with 4 TMFCs in Manual/Cascade Mode



1	**Manual/Cascade** mode has been selected; the button is green.
2	**NOTE:** Gas flow limits are 2-100 CCM (SLPM), not percentages.
3	The Totalizer feature tracks and displays the accumulated flow for the associated loop; here it measures accumulated air flow.
4	Use the **Reset Totalizer** button at any time to return to 0.0 and begin counting again.

NOTE:
To reduce the risk of clogging the microsparge, gas flow should not be set below +2 CCM.

Figure 33b: Sample Cascade Screen with 4 TMFCs

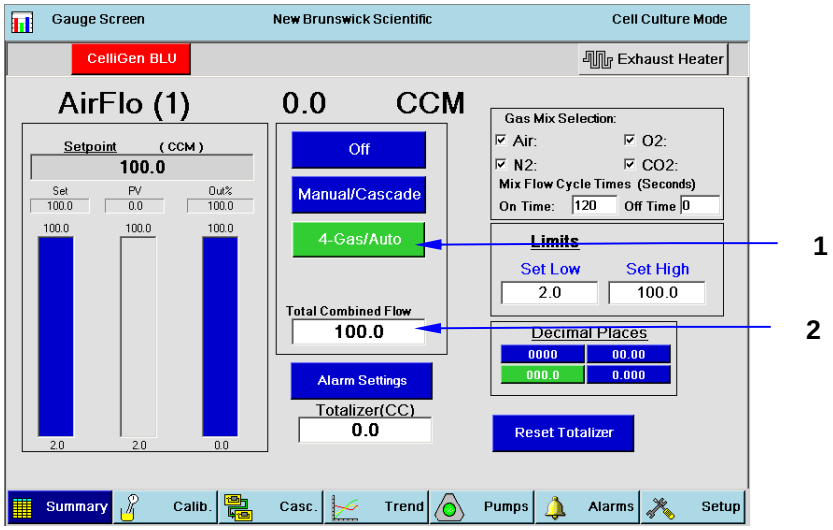


1	Press each loop's **Enable** button to activate automatic cascade control.

Cascade values are automatically controlled but first they need to be activated by selecting each loop's **Enable** button in the **CASCADE** screen (*see Figure 33b*). Default values are provided as starting points; they may need to be modified for the optimization of individual processes.

When you set the system to the **4-Gas/Auto** control mode (*see Figure 33c on the next page*), the controller automatically adjusts the flow ratio of Air, O_2, CO_2 and N_2 based on the demands of pH and DO. The individual flow rates for each gas change, but the total gas flow is maintained at a constant level set by adjusting the **Total Combined Flow**.

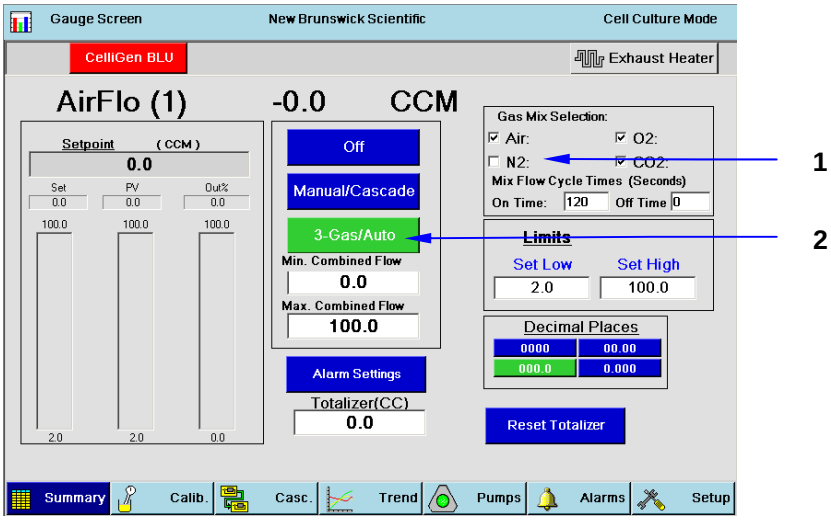
Figure 33c: AirFlo(1) Gauge with 4 TMFCs in 4-Gas/Auto Mode



1	**4-Gas/Auto** mode has been selected; the button is green.
2	Total Combined Flow box

If N_2 is deselected from the **Gas Mix Selection** pane, the system responds by changing to **3-Gas/Auto** mix. By changing to this mode, users now have the ability to define a minimum and maximum combined flow cascade as if they had a 3 TMFC system (*see Figure 33c and Section 11.3 above*).

Figure 33d: AirFlo(1) Gauge with 4 TMFCs in 3-Gas/Auto Mode



1	N2 has been deselected…
2	…and the Gas Control Mode has changed to **3-Gas/Auto**.

11.5 Gas Overlay Mixing

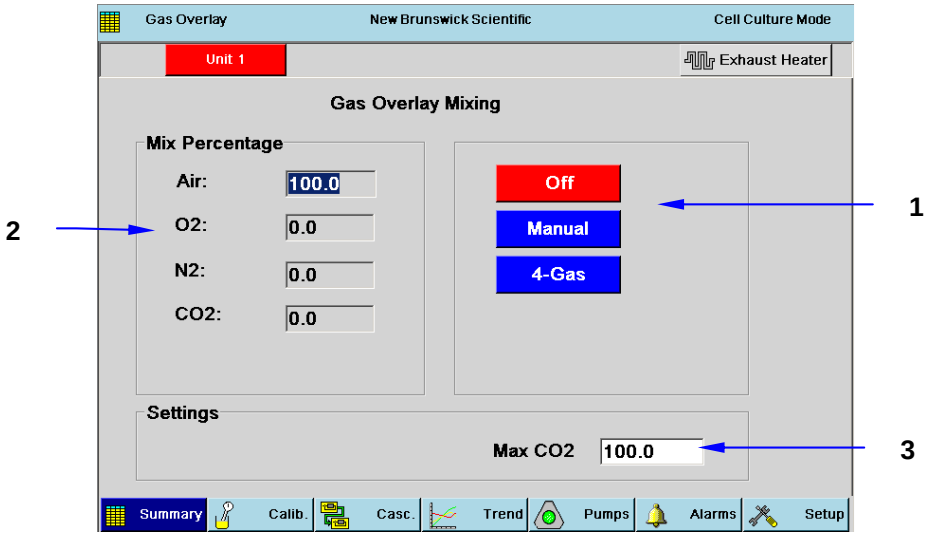
When the Gas Overlay option is present and set to Automatic Flow Control with at least one TMFC in the system, two additional process loops will be present in the **SUMMARY** screen: OvlMix (Gas Overlay Mix) and OvlFlo (Overlay Gas Flow).

If no TMFC is present, only the OvlMix process loop will be available and the flow must be controlled manually by means of a rotameter.

We recommend that you always use an overlay set to 4-Gas mix to help maintain your setpoints. It is especially important early in the run when there is little demand from the cultures.

Open the OvlMix loop gauge screen (*see Figure 34*) to set the parameters for the Gas Overlay option:

Figure 34: OvlMix Gauge Screen



1	Step 1: Set the operating mode for the Gas Overlay by pressing the button of choice (see Table 5).
2	Step 2: If you have selected Manual mode, use these edit boxes to set the percentage for O2, N2 and CO2 as desired; the system will automatically set Air so that all gases total 100%.
3	Step 3: If desired, use the Max CO2 edit box to set a maximum CO2 percentage. This is for 4-Gas mode only.

Table 5: Gas Overlay Operating Modes

Operating Mode	Description
Off	Press this button to turn the Gas Overlay option off.
Manual	Press this button to program the mix percentage of each gas yourself. *See Figure 34, Step 2.*
4-Gas	Press this button to leave automatic gas mixing to the controller. This selection disables user input to the Mix Percentage pane (*see Figure 34*).

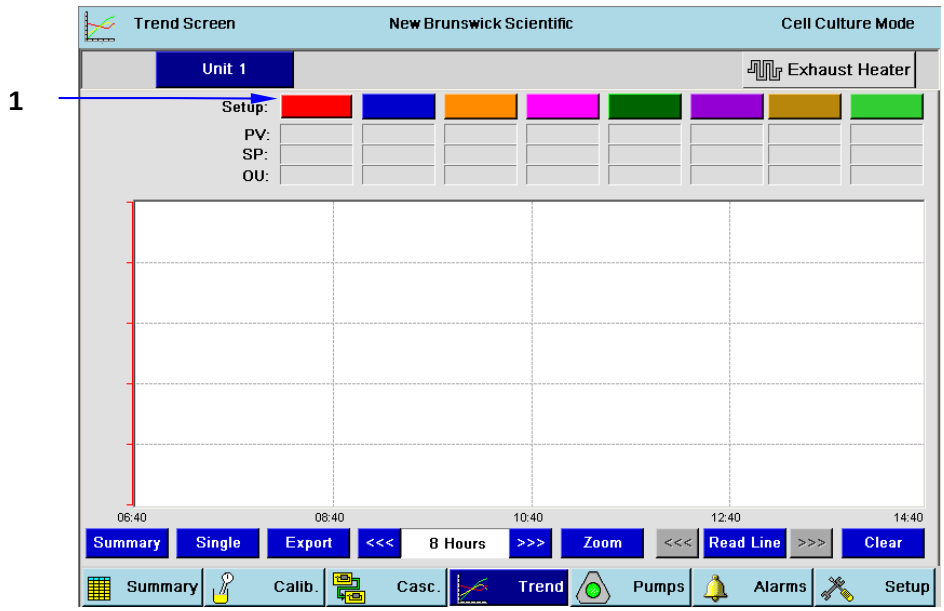
12 PLOTTING TRENDS

Opening the **TREND** screen allows you to plot and display a graph of ongoing culture data, viewing from 30 minutes to 144 hours of input. Up to 8 loops can be plotted on the graph, each in its own distinctive user-selected color. The graph and data are only available while the bioreactor is running. Data cannot be stored in the controller, but can be archived remotely on an auxiliary PC via the RS232/422 Modbus interface (*see Section 4.8 for details*) or saved to a USB storage device.

12.1 Creating a Trend Graph

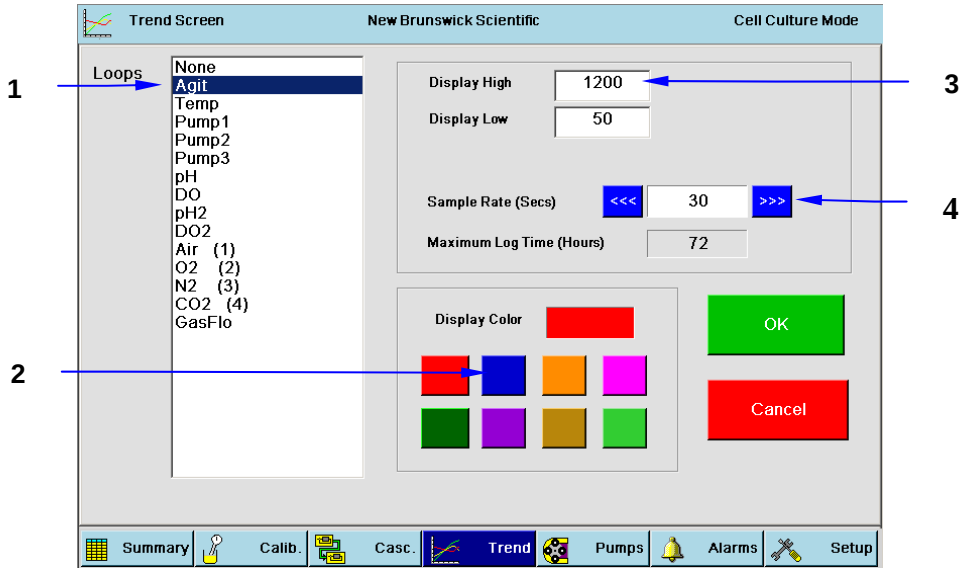
1. From any screen, press the **TREND** button to open the **TREND** screen (*see Figure 17*, repeated below for easy reference).

Figure 17: Trend Screen



1	Step 2: To select the first loop you wish to display, press the red **Setup** button.

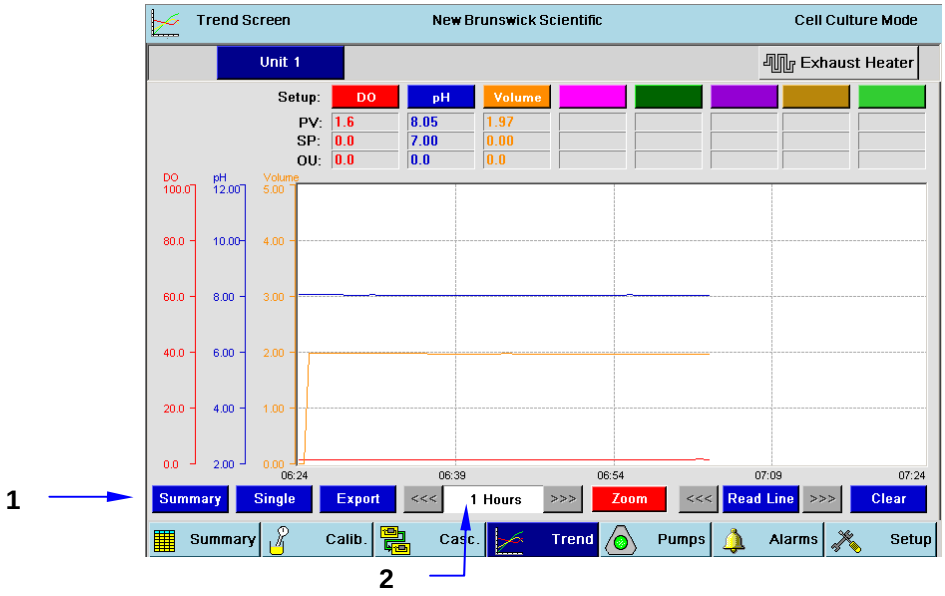
Figure 35: Trend Setup Screen



1	Step 3: Select the first loop. The program will automatically place it in the red box.
2	Step 4: If you wish to change the color of this loop, press the new color choice here.
3	Step 5: Press the Display High box to enter (using the touchpad) the high limit for the Y axis, then use the Display Low edit box to set the low limit.
4	Step 6: Press the ramp up >>> or ramp down <<< button to select the desired data sampling interval, 5, 15, 30 or 60 seconds.

7. Press **OK** to save your choice and return to the **TREND** screen, or Cancel to return to the **TREND** screen without saving any changes.
8. Repeat Steps 2 –7, selecting a different color for each loop, up to a maximum total of 8 loops.
9. With reference to Figure 36 (a sample Trend Graph in progress) and Table 6, acquaint yourself with the Trend Graph buttons at the bottom of the graph.

Figure 36: Trend Graph



1	Trend Graph buttons (see Table 6 for more information)
2	Timespan Indicator

Table 6: Trend Graph Buttons

Button/Feature	Description
Summary	Press this button to cycle through three summary display modes: all eight loops at once, loops 1-4 (red-fuschia), and loops 5-8 (dark green-light green).
Single	Press this button to display the graph for one loop at a time, in the order (left to right) they are displayed in the colored buttons at the top of the screen.
Export	Press this button to export a text file containing all of the Trend data. *See Section 12.1.1 for detailed instructions.*
<<< Ramp Down	Press this button to select a lower Timespan or to move the Read Line toward the left of the screen.
[Timespan Indicator]	Using the Ramp Down or Ramp up button on either side of this edit box, select the timespan to display onscreen. Preset increments range from 30 Minutes to 144 Hours.
>>> Ramp Up	Press this button to select a higher Timespan or to move the Read Line toward the right of the screen.
Zoom	Press this button to open an interactive mode where you can zoom in on a section of interest on one plot. The button turns red when you touch it, indicating you are in Zoom mode. *See Section 12.1.2 below for detailed instructions.*
Read Line	Press this button to open an interactive mode where you can move a vertical (cross-sectional) line across the graph to aid in determining a particular reading. *See Section 12.1.3 below for detailed instructions.*

12.1.1 Using the Export Button

To export Trend data as a text file to a USB external memory device for use with a PC program (e.g., Microsoft Excel®):

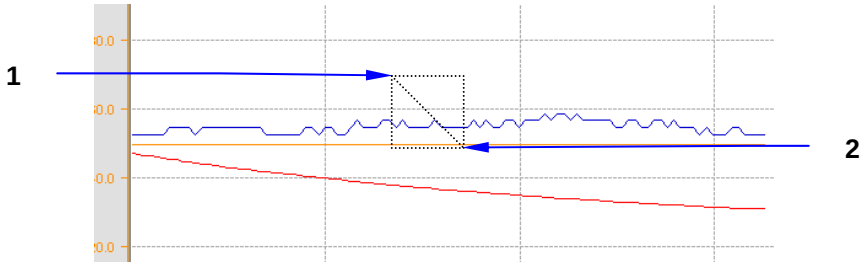
1. Install the USB external memory device into one of the USB connections on the back of the Control Cabinet.
2. Open the **TREND** screen and push the **Export** button.
3. In the screen that opens, select the USB external memory device from the list of available drives.
4. Touch the empty **FileName** box. Using the touchpad that appears, enter the desired file name, then press the **OK** button.
5. Press the **Save** button to save the file to the USB external memory device.
6. Remove the USB external memory device and use it to download the data to your PC.

12.1.2 Using the Zoom Button

To zoom in on a particular section of one loop plot:

1. Press the Zoom button at the bottom of the **TREND** screen. It will turn red to indicate that the zoom mode is active.
2. Press, in succession, two diagonal locations that would frame, left to right, the section of interest (*see Figure 37 for reference*):

Figure 37: Selecting Zoom Coordinates



1	Press first here (in the upper left corner of desired quadrant)
2	Press second here (lower right corner of desired quadrant). **NOTE:** the rectangle does not appear onscreen; it is indicated here for reference purposes only.

3. The Trend view will display the data between the two points selected, and will adjust the time axis to match the elapsed time represented by this close-up.
4. Press the Zoom button again to return to the regular trend graph.

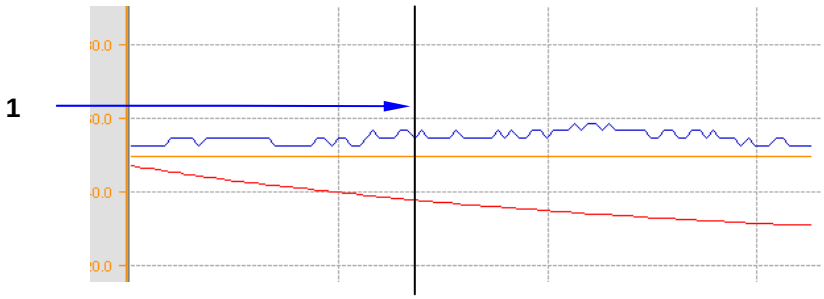
Minimum axis time in zoom mode is 120 seconds. If you wish to use the zoom mode and the read line (*see Section 12.1.3*) at the same time, you must enter Zoom mode first.

12.1.3 Using the Read Line

The Read Line mode allows you to read PV values from the graph (displayed at the top of the screen) at a position you select. To use the Read Line:

1. Press the Read Line button at the bottom of the **TREND** screen. It will turn red to indicate that the read line mode is active, and black vertical line will appear at the current time position on the graph.
2. To move the line to a time of your choosing, press the graph at the desired point. You can also press the Read Line <<< or >>> button (both are now red and active) to move the line one click at a time for more precision (*see Figure 38*):

Figure 38: Selecting a Read Line Location



1	Press anywhere along the desired vertical axis to locate the read line.

3. Press the Read Line button again to return to the regular trend graph.

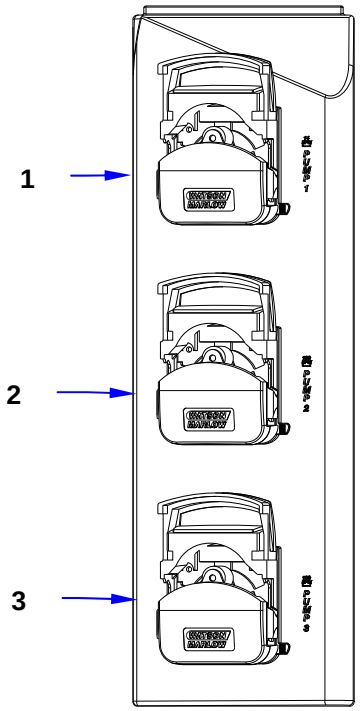
If you wish to use the zoom mode (*see Section 12.1.1*) and the read line at the same time, you must enter Zoom mode first.

13 ABOUT PUMPS

After assigning the pumps (*see Section 9.5*), you will need to select a setpoint and a control mode for each, calibrate their flow rates, and select their pulse periods. This section will walk you through those operations.

There are three standard pumps on the front right of your control cabinet (*see Figure 23*, repeated here for easy reference). Remember to set up any optional pumps you may have added to your system (*see Section 13.5 to install an external Barnant VS pump, and see Section 0, Appendix A, about optional stacked pumps*).

Figure 23: Standard Pump Array (pumps open)



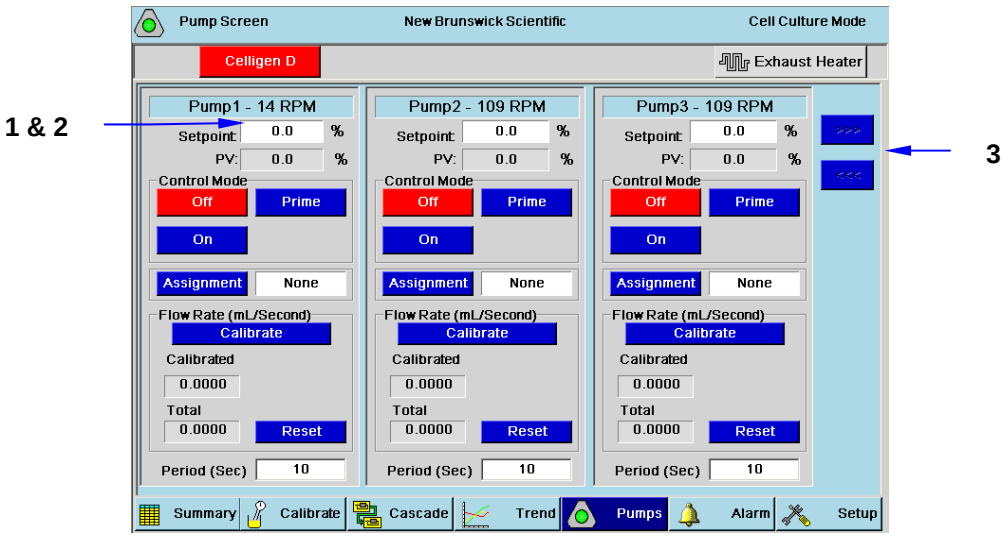
1	Pump1: 14 RPM	2	Pump2: 109 RPM	3	Pump3: 109 RPM

13.1 Pump Setpoint

To enter a setpoint for any pump:

1. Open the **PUMPS** screen. Gauges for Pumps 1-3 are displayed in this screen (*see Figure 39*). If you have one or more additional pumps, press the >>> button to continue past Pump 3. If you have a stacked pump, it will be governed by settings for its base pump.

Figure 39: Setting Pump Setpoint



1	Step 2: Press inside the Setpoint edit box for Pump1.
2	Step 3: Use the touchpad that pops up to enter the desired setpoint, then press the **OK** button in the touchpad to save the setpoint and to return to this screen (or press the **Cancel** button to return to this screen without saving a setpoint).
3	If optional pumps are installed on your system, these ramp up (**>>>**) and ramp down (**<<<**) buttons will be active, allowing you to scroll to the next page or back.

4. Repeat Steps 2-3 for each pump.

13.2 Pump Control Mode

There are three available control modes for each pump, as explained in Table 7:

Table 7: Pump Control Modes

Control Mode	Description
Off	The pump will receive no input and will not operate.
On	The pump will operate according to the parameters you have set.
Prime	This button toggles the pump on or off manually: as long as you press the button, the pump will run continuously. When you release the button, the pump will stop running.

NOTE:
If pumps are linked to a cascade, this may affect the ability to manually change setpoints and control modes.

To enter select a Control Mode for any pump, press the appropriate button in the Control Mode pane of the **PUMPS** gauge screen (*see Figure 39*).

13.3 Pump Flow Rate & Calibration Methods

The pump will always run at the same speed, but their flow rate depends on the diameter of the tubing you use. Table 8 provides the pump flow rates according to various tubing diameters.

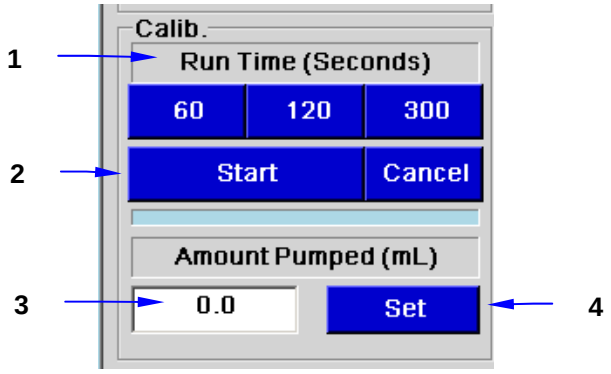
Table 8: Flow Rate per Tubing Size

Tubing Wall Thickness	1/16 inch (1.6mm)				
Tubing ID: inch (mm)	**1/32 (0.8)**	**1/16 (1.6)**	**1/8 (3.2)**	**1/4 (6.4)**	**5/16 (8.0)**
14 RPM Flow ml/minute	0.84	3.50	11.90	42.00	56.0
109 RPM Flow ml/minute	6.54	27.25	92.65	327.00	436.0

To calibrate any pump with the tubing you have selected:

1. Load approximately three feet of the tubing into the pump head.
2. Set up a reservoir with water at the input end of the tubing and an empty graduated cylinder, capable of measuring small quantities, at the output end of the tubing.
3. **Read this step completely before you do it:** with the input end of the tubing in the water reservoir, prime the tubing line by pressing the pump's **Prime** button, but allow it to run only until liquid **starts** to flow into the tubing: **DO NOT** allow the liquid to run into the graduated cylinder yet.
4. *If you are not using a scale, skip to Step 5.* If you are using a scale, place the graduated cylinder (with the tubing) on the scale and press Zero on the scale.
5. In the Flow Rate pane of the **PUMPS** screen (*see Figure 39*) for that pump, press the **Calibrate** button to open the Calibration pane (*see Figure 40*):

Figure 40: Calibrating the Pump Flow Rate



1	Step 6: Press your choice of Run Time (60, 120 or 300 seconds); that button will turn green.
2	Step 7: Press the Start button. The button will turn green and the pump will start running.
3	Step 8: When the Run Time has elapsed, record the amount (mL) of liquid accumulated in the cylinder, then enter that number (or the number registered on the scale) in the Amount Pumped edit box.
4	Step 9: Press the Set button to save this data to the PUMPS screen.

NOTE:
Calibration must be performed at operating setpoint.

NOTE:
Each pump and each tubing size will need its own calibration.

The pump is now calibrated. As the pump runs, you will see that the total will increase by this calibration standard.

13.4 Pump Period

At the bottom of each pump gauge is the Period (Sec) pane (*see Figure 41*):

Figure 41: Pump Period (Sec)

Use this edit box, and its associated touchpad, to enter a pump cycle time in seconds. For example, if the pump setpoint is 30%, setting a period of 5 seconds (as illustrated) will cause the pump to run 1.5 seconds, stop for 3.5 seconds, then cycle back on again.

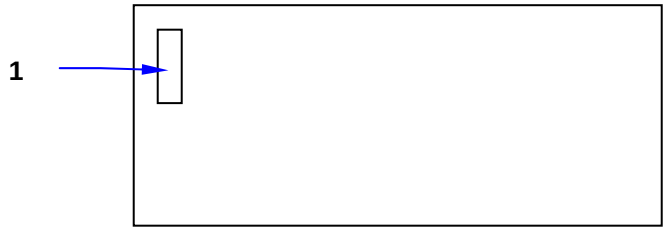
NOTE:
Running at a very low percentage renders the totalizer's results inaccurate. We recommend the use of smaller tubing to avoid choosing a very low percentage for the pump setpoint.

13.5 Installing an External Variable Speed Pump

CAUTION!
When selecting an external pump to operate with your system, please consult with your local sales representative to be sure the model you choose is compatible with your CelliGen BLU.

1. Connect the D connector of the pump's 3-wire cable at **Interface** on the rear panel of the pump (*see Figure 42 on the following page*). Your pump will be marked 115V or 230V, depending on the electric supply you specified when you purchased the pump.

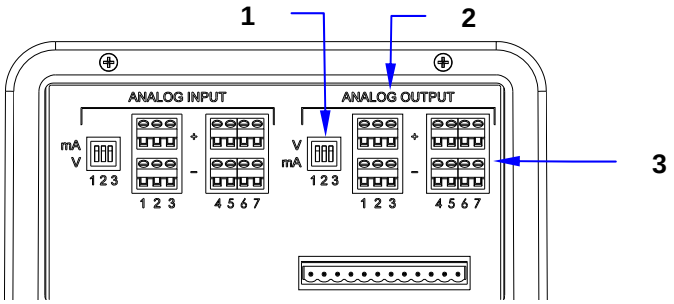
Figure 42: Variable Speed Pump



1	Use **Interface** to connect the cable provided.

2. Locate the Analog Output Connections on the rear of the CelliGen BLU cabinet (*see Figure 43*).

Figure 43: Rear Panel of CelliGen BLU Cabinet



1	These dip switches are provided to switch connectors 1-3 between mA (down) and V (up)		
2	These dip switches (1-3) are for mA or V	3	These dip switches (4-7) are for V only.

3. The preferred connection for pumps is 4-20mA. *If you are using a 0-5V connection, skip to step 5.* To use 4-20mA, connect the end of the green cable wire to one of the three (1, 2 or 3) negative (-) **outputs** at the bottom.

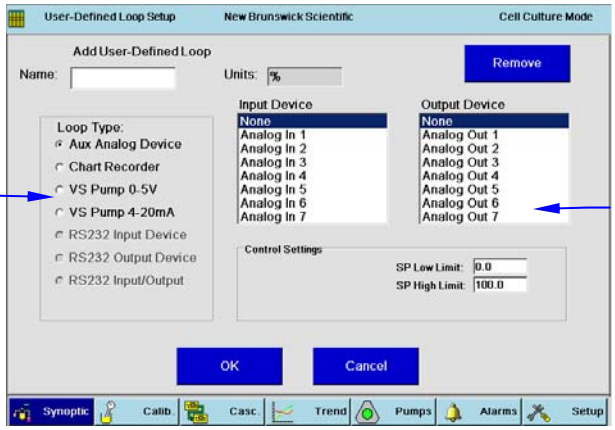
4. Connect the end of the white cable wire to the positive (+) output at the top: *be sure to use the same number* (1, 2 or 3) as you used for the green wire.

5. If, *and only if*, you are using a 0-5V connection instead of 4-20mA, connect the end of the green wire (return) to one of the four (4, 5, 6 or 7) negative (-) outputs at the bottom and connect the end of the black wire (0-5V input) to the matching positive (+) outputs at the top.

6. Set up the pump control loop using a loop for external equipment (*see Section 6.2.6 & Figure 11, repeated on the following page for easy reference*).

After a pump is added, it will appear on the **PUMPS** screen.

Figure 11: Add User-Defined Loop Screen



1	Selections for variable speed (0-5V and 4-20mA) pumps.
2	Each TMFC in the system will utilize one of the 0-5V Input/Output devices on the board. If, for example, this system was configured with 1 TMFC that was assigned as I/O Device 4, you would find I/O4 to be missing from the Input Device & Output Device lists.

CAUTION!

Be sure to set the dip switches correctly when using either 4-20mA or 0-5V inputs/outputs.

14 ABOUT ALARMS

14.1 ABS and DEV Alarms

There are two types of alarm modes you can set, Absolute (ABS) and Deviation (DEV):

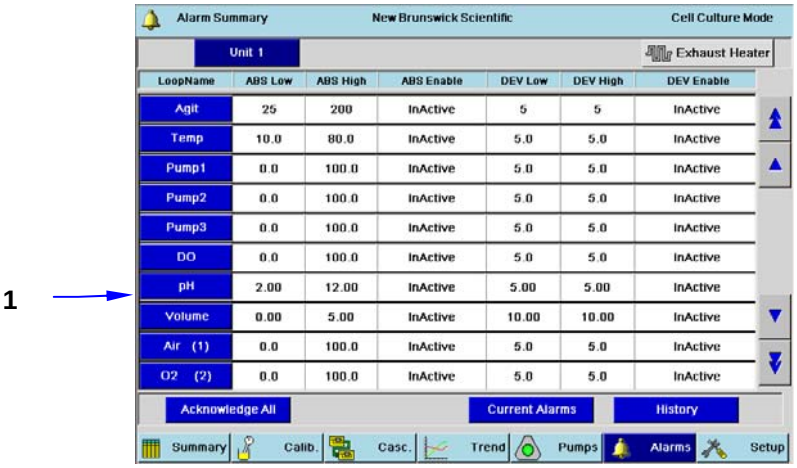
- An Absolute alarm is triggered when the control loop's Process Variable falls below the absolute Low limit or rises above the absolute High limit that you set.
- A Deviation alarm is triggered when the control loop's Process Variable falls below or rises above the control band that you specify around the loop's setpoint (e.g., a tolerance of 10 rpm above or 5 rpm below the Agitation setpoint).

14.2 Setting Alarms

To set alarms:

1. Press the **ALARMS** button to open the **ALARMS** screen (*see Figure 19*, repeated here for easy reference, *and Table 9*, which explains the features of this screen).

Figure 19: Alarms Screen


Alarm Summary
New Brunswick Scientific
Cell Culture Mode
Unit 1
Exhaust Heater
LoopName | ABS Low | ABS High | ABS Enable | DEV Low | DEV High | DEV Enable
Agit 25 200 InActive 5 5 InActive
Temp 10.0 80.0 InActive 5.0 5.0 InActive
Pump1 0.0 100.0 InActive 5.0 5.0 InActive
Pump2 0.0 100.0 InActive 5.0 5.0 InActive
Pump3 0.0 100.0 InActive 5.0 5.0 InActive
DO 0.0 100.0 InActive 5.0 5.0 InActive
pH 2.00 12.00 InActive 5.00 5.00 InActive
Volume 0.00 5.00 InActive 10.00 10.00 InActive
Air (1) 0.0 100.0 InActive 5.0 5.0 InActive
O2 (2) 0.0 100.0 InActive 5.0 5.0 InActive
Acknowledge All
Current Alarms
History
Summary
Calib.
Casc.
Trend
Pumps
Alarms
Setup

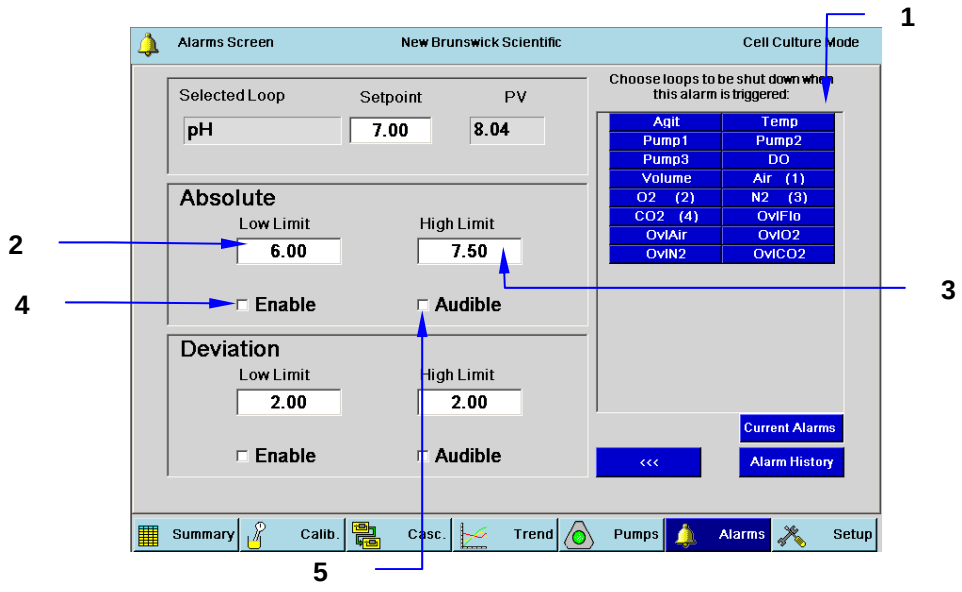
1

1	Step 2: Press the first loop for which you want to enable an alarm. That loop's individual Alarms Screen will open. For this example (see Figure 44) we use the pH loop. The instructions continue after Table 9.

Table 9: Alarms Screen Features

Feature Name	Description
LoopName	Like the CelliGen BLU name box, this box is blue under normal operating conditions, and red when there is an alarm condition. Press a LoopName box to open that control loop's alarm screen.
ABSLow	This column indicates the Absolute low limit you program for control loops. An alarm is triggered if the loop PV falls below this point.
ABSHigh	This column indicates the Absolute high limit you program for control loops. An alarm is triggered if the loop PV rises above this point.
ABSEnable/ABSAudible	This column indicates whether the Absolute alarm limits have been enabled ("Active") or not ("InActive") for visible (ABSEnable) and/or audible (ABSAudible) alarms.
DEVLow	This column indicates any tolerance you have set below the control loops' setpoints.
DEVHigh	This column indicates any tolerance you have set above the control loops' setpoints.
DEVEnable/DEVAudible	This column indicates whether the Deviation alarm limits have been enabled ("Active") or not ("InActive") for visible (DEVEnable) and/or audible (DEVAudible) alarms.
Acknowledge All button	Press this button to acknowledge (and stop) all alarms.
Current Alarms button	Press this button to open a screen that addresses any current alarm condition.
History Button	Press this button to open the historical record of alarms for the current run.
Scroll Up (ϖ) or Scroll Down (ω)	Use this button to scroll upwards or downwards in the table onscreen.
Scroll Back (<)	Use this button to return to a previous screen.

Figure 44: Sample Loop Alarms Screen (pH)



1	Pressing any of these loop buttons will cause the selected loop(s) to shut down when an alarm is triggered for the loop whose Alarms screen this is (in this case, pH).
2	Step 3a: If you wish to set an Absolute alarm, enter an Absolute Low Limit here…
3	Step 3b: …and enter an Absolute High Limit here.
4	Step 4a: Press the Enable checkbox to enable the Visual alarm…
5	Step 4b: …and check the Audible checkbox to enable the Audible alarm.

5. If you wish to set a Deviation alarm, use the **Deviation** pane and follow the same procedure as outlined in Steps 3a-4b above.
6. Use the **Scroll Back** button (**<<<**) to return to the main Alarms screen and follow these steps for any other alarms you wish to set, or press the **Summary** button to return to the **SUMMARY** screen.

14.3 Acknowledging an Alarm

When an alarm condition develops, the LoopName box on the **SUMMARY** screen for the control loop involved will turn from blue to red, as will the CelliGen BLU name box. This is the Visual alarm. A footnote, written in red, will also appear in order to identify the nature of the alarm (e.g., Unit 1—Deviation Low Error).

The Visible alarm will remain onscreen until the alarm condition is rectified. If the Audible alarm is also enabled, beeping will occur until the alarm is acknowledged.

There are three ways to acknowledge alarms: (1) one alarm at a time, (2) all alarms for one control loop at a time, and (3) all alarms for all control loops at a time, for the rare occasion such a condition should arise.

To acknowledge **one alarm at a time**:

1. Press the **ALARMS** screen button to open the **ALARMS** screen.
2. Press the red **LoopName** box to open that control loop's **ALARMS** screen.
3. Press the **Current Alarms** button to open the **Current Alarms Summary** screen.
4. Press the Index box for the alarm you wish to acknowledge. It will turn green.
5. Press the **Acknowledge** button. The alarm will be deleted from the screen.
6. Repeat Steps 4 & 5 for any other alarms recorded for this loop.
7. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.
8. Repeat Steps 2-7 for any other control loop alarms.

To acknowledge **all alarms simultaneously for one control loop**:

1. Press the **ALARMS** screen button to open the **ALARMS** screen.
2. Press the red **LoopName** box to open that control loop's **ALARMS** screen.
3. Press the **Current Alarms** button to open the **Current Alarms Summary** screen.
4. Press the **Acknowledge All** button. All alarms will be deleted from this screen.
5. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.

To acknowledge **all alarms for all control loops** at the same time:

1. Press the **ALARMS** screen button to open the **ALARMS** screen.
2. Press the **Acknowledge All** button. All alarms will be deleted from this screen.
3. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.

CAUTION!

Acknowledging alarms is NOT a replacement for correcting the condition that triggered the alarm. Diagnose the cause of the alarm condition and rectify the situation to ensure proper operation of your CelliGen BLU.

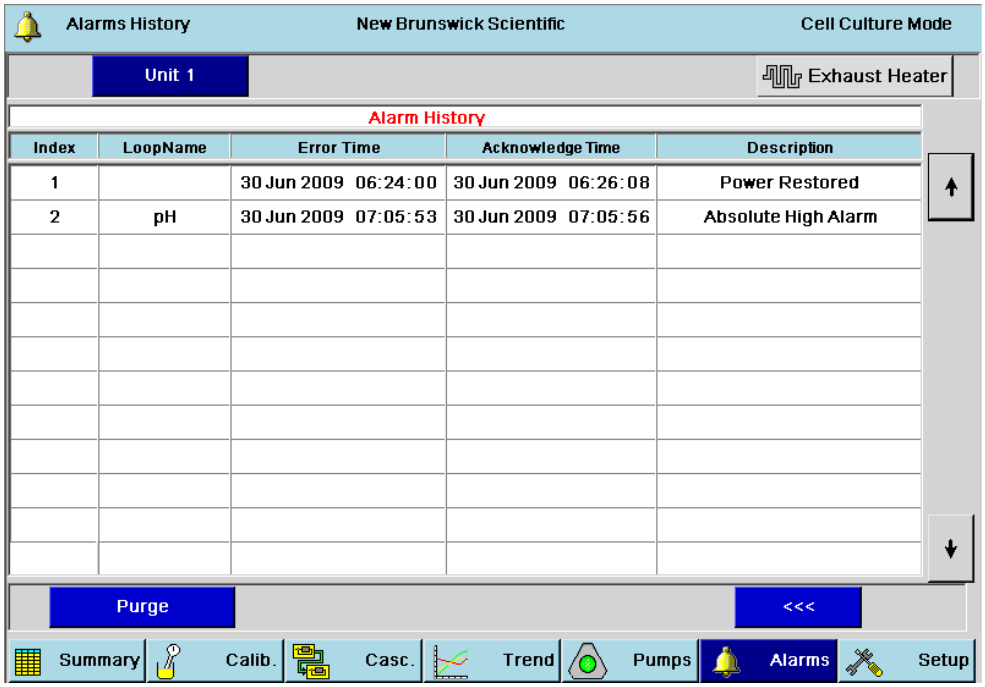
14.4 Alarms History

Each time an alarm is triggered, whether Visible and/or Audible, the controller records the event. The controller also records each alarm acknowledgement. You can access the Alarms History screen (1) to consult the data, (2) to save the data to an optional auxiliary PC, and/or (3) to purge the records once the condition has been rectified.

To access the Alarms History screen (*see Figure 45 on the following page*) **to consult data**:

1. Press the desired control loop's **LoopName** box in the main **ALARMS** screen.

Figure 45: Sample Alarms History Screen



2. In the control loop's **Alarms Screen** that opens, press the **Alarm History** button.
3. Press the Scroll Down (ω) or Scroll Up (ϖ) button to read through the data.
4. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.

To access the Alarms History screen **to purge the history**:

1. Press the desired control loop's **LoopName** box in the main **ALARMS** screen.
2. In the control loop's **Alarms Screen** that opens, press the **Alarm History** button.
3. Press the **Purge** button to erase all records.

NOTE:
You cannot delete one record at a time; you can only purge all records simultaneously.

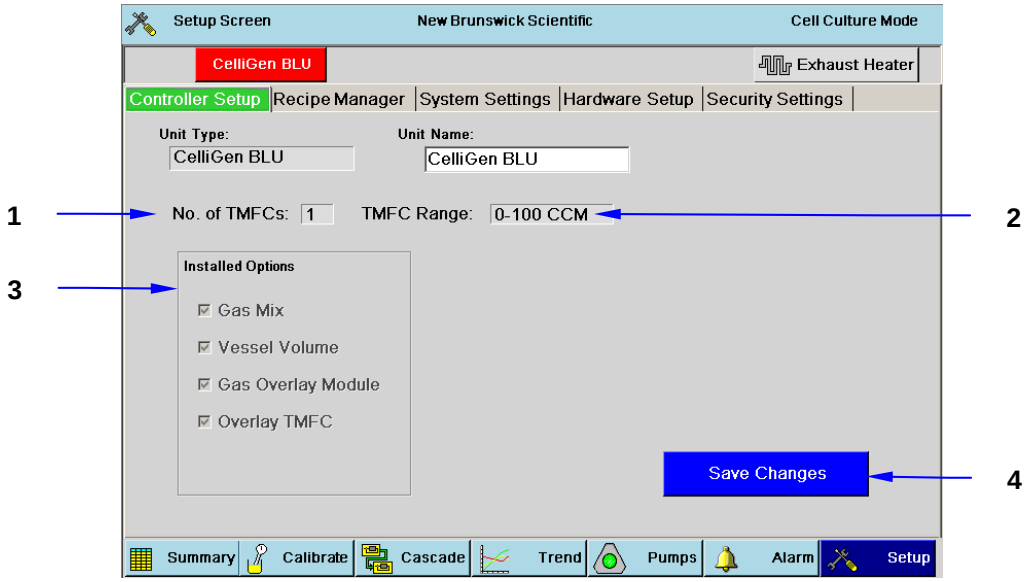
4. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.

15 USING THE SETUP SCREEN

The **SETUP** screen (*see Figure 20a, repeated below for easy reference*) has one feature that you will use with frequency, the **Recipe Manager** (*see Section 15.2*).

You can also use this screen to change **Controller Setup** (*see Section 15.1*), to adjust **System Settings** (select onscreen language when available, change date & time, update software and calibrate the touchscreen; *see Section 15.3*), and to check or change the **Hardware Setup** (*see Section 15.4*).

Figure 20a: Setup Screen



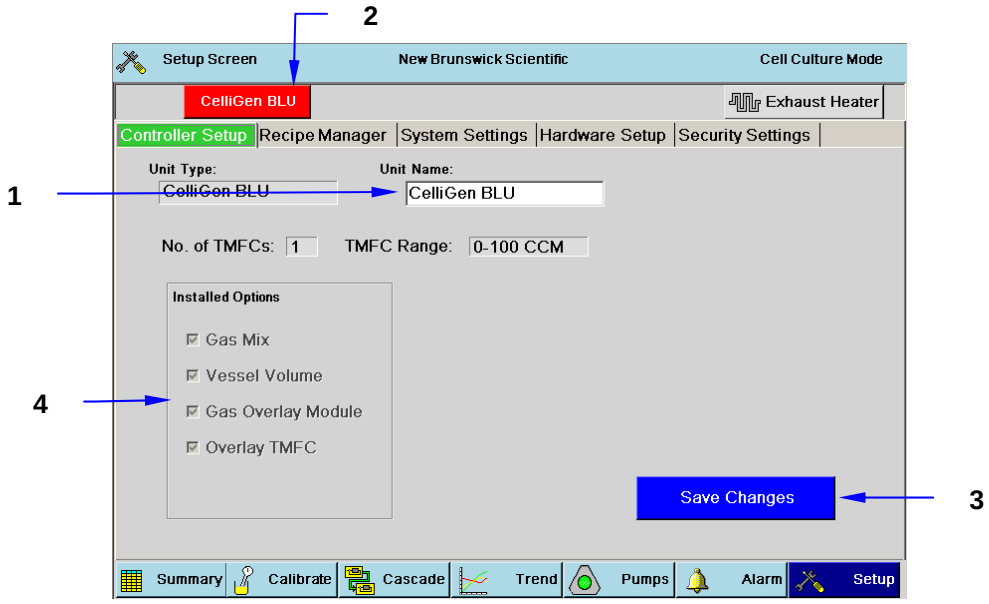
1	The quantity of Thermal Mass Flow Controllers (TMFCs) installed
2	The flow range of your TMFC(s)
3	The factory-installed options present on your system
4	Whenever you make a change, be sure to press the **Save Changes** button.

In addition, you may wish to access this screen, if you need to contact NBS Customer Service about your CelliGen BLU, to check the status of installed equipment and the firmware version (which you also see briefly in the **START-UP** screen) by consulting the **Hardware Setup** pane.

15.1 Controller Setup

When you open the **SETUP** screen, normally the **Controller Setup** screen will display first (*see Figure 20a above and on the following page*). If you find any other Setup screen in the display, press the **Controller Setup** tab to open this screen.

Figure 20a: Controller Setup Screen



1	Press inside the Unit Name edit box to open a touchpad. The name you write in this box will appear on a colored button tub on the top menu line.
2	Step 2: The new Unit Name button tab appears here.
3	Step 3: Press the Save Changes button to save the new Unit Name.
4	Installed Options: The controller automatically detects all factory-installed options present and reflects them here with checks in the appropriate checkboxes (☑).

Name the bioreactor using the Unit Name edit box touchpad. You may wish to simply name the unit by its default designation in the **Hardware Setup** screen (*see Section 15.4*): Unit 1.

The **Save Changes** button saves your new Unit Name. If you leave this screen and wish to save any change you made, be sure to press the **Save Changes** button *before* you move to another screen.

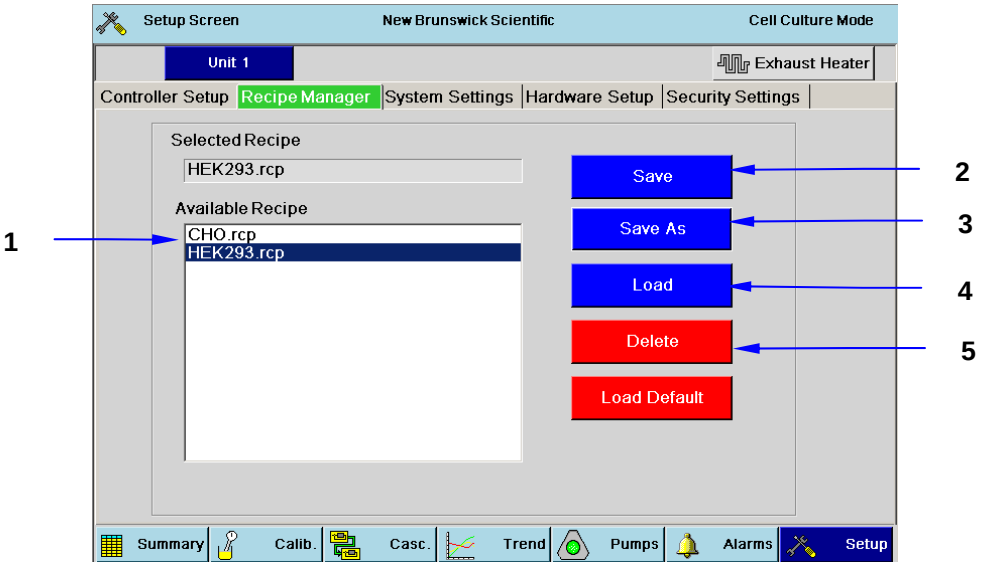
15.2 Recipe Manager

Press the second tab in the **SETUP** screen to open the **Recipe Manager** screen (*see Figure 20b, repeated on the following page*). Use this feature to access, rename, save, load and delete recipe files for your cell culture runs.

Recipes consist of all user-definable variables available on the **CONTROL** screens. When a recipe is saved, all the current settings on the controller (including but not limited to setpoints, control modes, alarms, P&I values, and cascades) are saved to the controller's memory.

You can save this data with a unique name using the **Save As** button (*see Step 2b below*), or overwrite an existing recipe using the **Save** button (*Step 2a*):

Figure 20b: Recipe Manager Screen



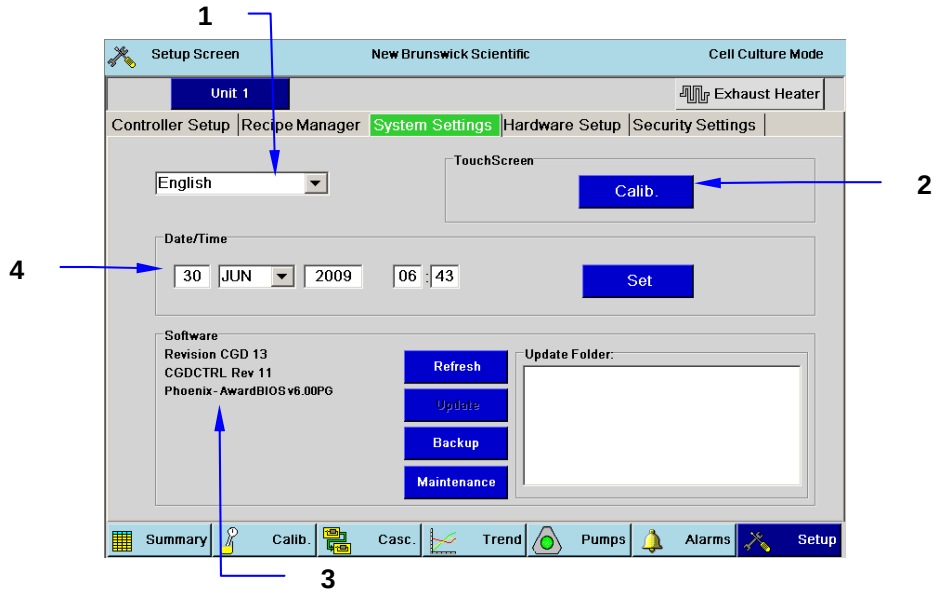
1	Step 1: Press the recipe file of choice in the list shown in this box.
2	Step 2a: Press the **Save** button to save the recipe as is, or…
3	Step 2b: Press the **Save As** button if you wish to rename the file; use the pop-up touchpad to designate a new name.
4	Step 3: Press the **Load** button to load the Selected Recipe file.
5	Step 4: To delete a recipe from the system, select it (see Step 1), then press the **Delete** button.

The controller is capable of storing up to 10 recipes. You can retrieve these recipes by opening the **Recipe Manager** screen, where all saved recipes are listed in the Available Recipes pane. Select the desired recipe by following Step 1 in Figure 20b, then load it as shown in Step 3.

15.3 System Settings

Press the third tab in the **SETUP** screen to open the **System Settings** screen (*see Figure 20c on the following page*). Use this feature select the onscreen language you prefer, to reset the date and/or time, to update the software, and to calibrate the CelliGen BLU touchscreen.

Figure 20c: System Settings Screen



1	Other languages are not available at this time.
2	To recalibrate the system's touchscreen, press the Calib. Button, then touch the onscreen target each time it appears. You will be guided through the process.
3	Listed here are the current User Interface and Control Program versions. To update the software, see Section 15.3.2.
4	To change the Date and/or Time, see Section 15.3.1.

15.3.1 Resetting Date/Time

To reset the onscreen date and/or time (displayed in the lower righthand corner of every screen):

1. In the System Settings screen press the edit box for the numeric parameter you wish to change.
2. Use the pop-up touchpad to input the new number and press the **OK** button.
3. To change the month, press the down arrow (▼) and press the month you wish to select from its associated drop-down menu.
4. Press the **Set** button to save the new information. You can do this after each change, or after all changes have been made.

15.3.2 Updating Software

To update the system software, obtain a new version of the software in a USB drive and plug the drive into the USB port on the control cabinet:

1. In the **System Settings** screen, press the **Refresh** button to update the current software status and to search for a new USB drive.
2. The name of the new drive folder appears in the **Update File** box.

3. Press the **Update** button to install the file. The file will reboot twice; this may take a little time.
4. The Software pane will reflect the changes.

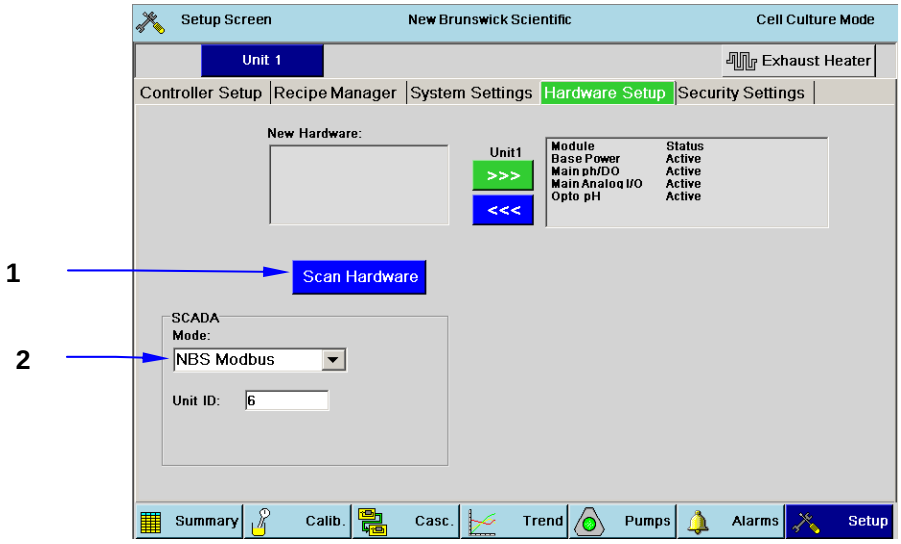
Updating software will not affect any previous user settings.

15.4 Hardware Setup

The CelliGen BLU system you purchased is preset in the factory as "Unit1" with all the accompanying hardware. In the Unit1 hardware list shown in the sample **Hardware Setup** screen (*Figure 20d below*), the system has the Base Power module, the Main pH/DO module, the Main Analog module and the Opto pH module.

This system is also set to Modbus communication mode (*see the SCADA pane in Figure 20d*), and has the Unit ID number of 6. This is the unit's multidrop identification number.

Figure 20d: Hardware Setup Screen



1	The Scan Hardware function is reserved for use only by authorized Service Technicians.
2	SCADA address

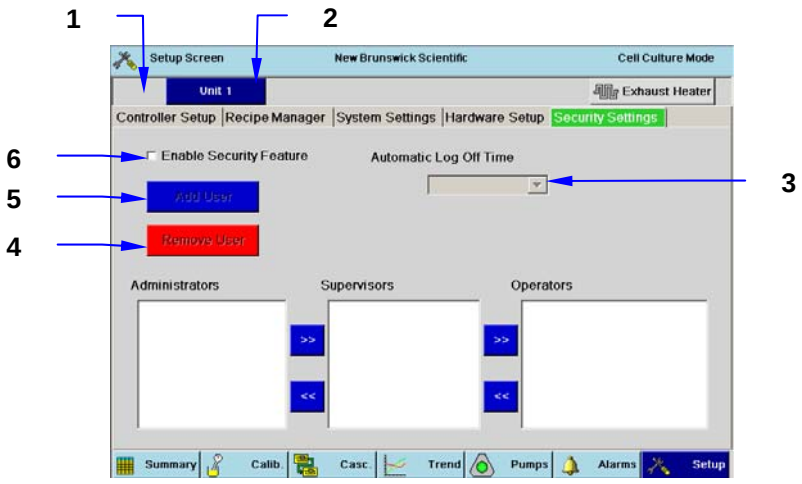
15.5 Security Settings

The security feature on the BLU provides two user access levels:

- **Operators** have access to routine operations but they change security settings.
- **Administrators** have access to all operations including defining new users (operators and administrators) and setting security parameters.

Press the fifth tab in the **SETUP** screen to open the **Security Settings** screen (*see Figure 46*):

Figure 46: Security Settings Screen

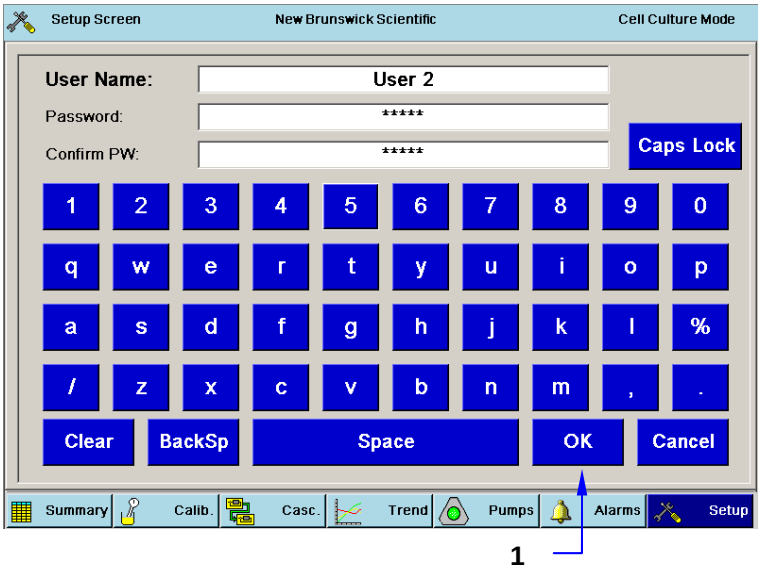


1	When security is enabled, the **User** button appears in this corner of all main screens.
2	This block will show the name of your unit.
3	Use the dropdown menu here to define the time before the system automatically logs off, leaving only the SUMMARY, SYNOPTIC and TREND screens available.
4	**Remove User** button (see text below)
5	**Add User** button (see text below)
6	Only a user in the Administrator group has access to this feature: checking "Enable Security Feature" turns security on; deselecting it turns security off.

In this screen, a user with Administrator status can move users from the Operators group to the Administrators group (or vice versa) by highlight the user name in the Administrators or Operators pane, then pressing the **>>** or **<<** button to move that user from one pane to the other.

An Administrator can also add users to or remove users from the system using the **Add User** and **Remove User** buttons. To remove a user, press the user name to select it in the pane where it appears, then press the **Remove User** button. To add a user, press the **Add User** button, use the keypad screen that opens (*see Figure 47 on the following page*) to type in the user name (and assign a Password if desired; if you add a Password, you will be prompted with "Confirm PW" to type it again to confirm it), then press the **OK** button.

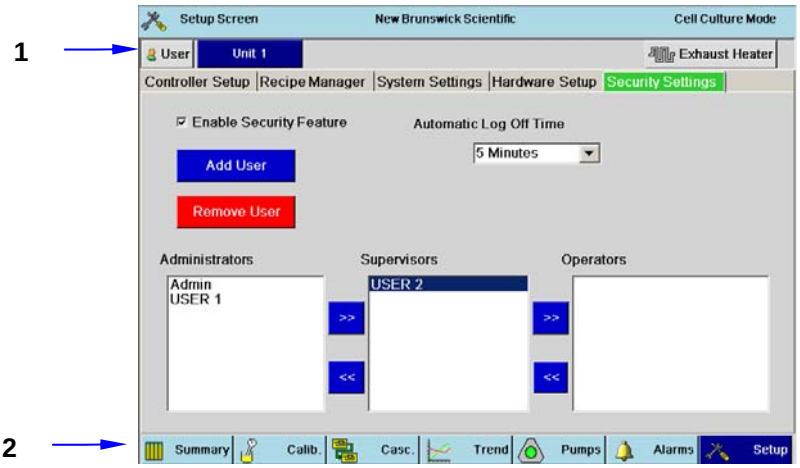
Figure 47: Security Keypad



1	When the User Name and Password are entered as desired, press the **OK** button.

As shown in the Security Settings Screen on the previous page and in the Summary Screen below, when security is enabled, the **User** button appears in the top left corner of all major screens.

Figure 48: User Button



1	When a user presses the **User** button, that user can use the popup buttons to **Log Off** or to **Log On** by pressing the appropriate button. If the user has Administrator status, the **Change Password** button will also be available. Pressing it will open the security keypad to change his/her password. **ADMIN** is the default password.
2	When Security is enabled, only the **Synoptic/Summary** and **Trend** navigation buttons remain active; the other navigation buttons will be greyed out and inaccessible.

16 PERFORMING A CELL CULTURE RUN

16.1 Preparing for Start-Up

1. Add sterile filtered media:
   - Aseptically connect the addition port tubing to the addition vessel (which contains the media). This can be done using a tube welder or by making the conection under a hood.
   - Open the addition tubing clamps.
   - Using gravity or a peristaltic pump, move the media into the vessel.
   - Close the addition tubing clamps.

2. Set temperature control to the desired working temperature.
3. Check that agitation is in **OFF** mode.
4. Connect the motor.
5. Set agitation to the desired speed.

CAUTION!

Agitation can only be increased 25 RPM at a time; any greater increase will cause the magnetic drive to uncouple.

6. Set mode to **AUTO**.
7. Draw a sample from the vessel (*see Section 16.4 for the sampling procedure*).
8. With an external pH meter measure the pH of the liquid, noting its pH and temperature.
9. Adjust the display reading of the pH function to read the value displayed on the external pH meter. Make a correction for the pH value if the temperature of the vessel is different from that of the sample. During prolonged cell growth it is advisable to take a sample from the culture and measure its pH with an external pH meter. If the readings are different, the pH sensor's zero has drifted. Readjust the zero on the Vessel pH function to match the reading of the external pH meter.
10. When the vessel reaches the desired working temperature, calibrate the DO probe *(see Section 7.3 & Section 7.4)*.

NOTE:

When the media temperature in the vessel is much lower than the desired working temperature, be careful not to set the temperature to the desired value at once as that could overheat the media.

…continued…

Generally, set the setpoint about 1°C below the desired working temperature first. As the temperature rises, reset the setpoint several times. Finally, the desired temperature can be set and the vessel temperature will reach its desired value without substantial overshoot.

16.2 Inoculation

1. All addition and harvesting vessels should be sterile. The addition vessel should also be siliconized for microcarrier culture.
2. Set Agitation and Temperature to **OFF**.
3. Turn the power **OFF**.
4. Inoculate the vessel in the following way:
   - Aseptically connect the addition port tubing to the addition vessel.
   - Aseptically place the sterile media and cells (and the sterile microcarriers, if you are using them; see important **NOTE** below) in the addition vessel.
   - Open the addition tubing clamps.
   - Dispense the contents of the inoculation vessel into the vessel by gravity or by using a peristaltic pump if appropriate to the working volume.

NOTE:
If microcarriers are being used, they should be placed in the addition vessel with cells and part of the media. After dispensing this into the vessel, the rest of the media should be placed in the addition vessel and dispensed into the vessel. This rinses microcarriers from the addition vessel and tubing.

   - Close the addition tubing clamps.
   - Aseptically disconnect the addition port tubing from the addition vessel.
5. Turn the power switch **ON**.
6. Set Agitation to the desired RPM and the mode to **AUTO**.

CAUTION!
Agitation can only be increased 25 RPM at a time; any greater increase will cause the magnetic drive to uncouple.

NOTE:
For microcarrier culture, the agitation should initially be set at 30-50 RPM. After the cells have attached to the microcarriers, the agitation can be increased above 50 RPM, depending upon the microcarrier and cell being used. For suspension culture, the speed should be 30-50 RPM.

7. Set the DO and pH to the desired control value.

8. Set the DO and pH mode to **AUTO**.
9. Connect the Gas Inlet tubing to the filtered Gas Delivery System being used.
10. Turn on the gases to 5 PSIG pressure.
11. Display gas screen, set mode to **3-GAS** or **4-GAS**, set cycle time.

NOTE:

If, at the very beginning of operation, the pH value of the media is higher than the setpoint by 0.3 or more, it is recommended to set the control mode to "Manual" and add sufficient percentage of CO_2 gas into the vessel to bring down the pH value. Then change mode to "4-GAS".

12. Adjust the airflow to the desired flow rate. It is best to start at the lowest airflow rate at which the unit can control pH and DO and increase the flow rate only when the unit can no longer control pH and DO at that flow rate. We recommend that you maintain pH and DO using an overlay, then using overlay and sparge.
13. Check to see that the flow is stable. Check to see that all the gases are connected properly.

16.3 Start BioCommand *(if present)*

If you are using *BioCommand* supervisory software:

1. Start *BioCommand* on your computer, reset the EFT ("Elapsed Fermentation Time") to zero, make appropriate program selections to begin logging data.
2. Make sure all gas pressures are 5 PSIG.
3. If your CelliGen BLU has rotameter air flow control, adjust the airflow to the desired rate. Check to see that flow is stable and that all gases are properly connected.

16.4 Sampling the Vessel

A sample port with C-Flex® tubing, a CLAVE® NeedleFree connector and Luer-Lok™ NeedleFree syringes are supplied as the sampling method for every vessel. Follow this procedure to draw a sample from your vessel:

1. Aseptically remove the cap from the CLAVE connector and swab the exposed area with sterile IPA.

2. Open a sterile NeedleFree syringe package and swab the Luer connector with IPA.

3. Attach the NeedleFree syringe to the CLAVE connector.

4. Open the tubing clamp is one is attached to the sample tubing.

5. Slowly draw the sample by pulling back the syringe plunger until the desired volume has been removed. You may need to use one syringe to purge the line of old media, then a fresh syringe to draw the sample.

6. Aseptically disconnect the syringe.

7. Swab the CLAVE connector again with IPA and reinstall the cap.

When you draw additional samples, we recommend that you flush the sample line by pulling a small sample first with one syringe to remove any old media and cells from the line (the standard sample tube holds approximately 5 ml of liquid), then draw a second sample with a fresh syringe to be your actual sample.

16.5 Shutdown

1. Turn off all gases.
2. Set the control mode of all loops to **OFF** and disconnect the agitation motor.
3. Turn the power **OFF**.
4. Disconnect the power plugs.
5. Disconnect the probe cables.
6. Remove the DO and temperature probes and store them properly *(see their respective manuals)*.
7. Aseptically harvest the contents of the vessel.
8. Clamp off all tubing connections.
9. Properly dispose of the entire vessel assembly, including the pH probe, respecting all appropriate precautions regarding biohazards.

17 CLEANING

At least once a month, clean all the metal parts of your control cabinet. Use a soft, damp cloth moistened with water or mild detergent. If a detergent is used, remove all residue by rinsing them with clean water.

When you clean the heat blanket, wipe it with a clean cloth moistened with isopropyl alcohol.

18 MAINTENANCE

Preventive maintenance keeps your equipment in proper working condition. When performed routinely, maintenance results in longer life for your equipment. It also reduces time lost due to equipment failure.

WARNING!
Always turn your CelliGen BLU off and disconnect the power cord before performing maintenance.

18.1 Probe Maintenance and Storage

Use soft facial tissue to clean the DO and temperature probes.

When they are not in use in a vessel, the DO and temperature probes should be stored. **At no time should either probe be allowed to rest on its tip.**

CAUTION!
Never let a probe rest on its tip.

18.2 Periodic Inspection

At three-month intervals, perform the following checks and inspections.

NOTE:
Before you begin, make sure that the power switch is in the OFF position and that the power supply has been disconnected.

1. Check all controls and accessible items (power switch, connectors, screws, nuts and bolts) to make sure they are properly tightened. Tighten any loose item(s).
2. Check that all controls and connectors are free of dust.

18.2.1 Motor Assembly Replacement

WARNING!
NO ONE BUT A PROFESSIONAL SERVICE PERSON should touch electric or electronic parts or assemblies in the control cabinet.

If the motor assembly should require replacement, call for an authorized NBS service technician.

18.3 Fuse Replacement

There is one replaceable 5-Amp fast-acting glass tube fuse.

19 SERVICE

If any problems occur with your CelliGen BLU system, do not attempt to perform any service on it. Unauthorized servicing may void the warranty. Please contact your local NBS Service Department or your local NBS distributor.

In any correspondence with NBS, please refer to the Model Number (CelliGen BLU), and the Manufacturing Part Number and Serial Number of the unit.

19.1 Troubleshooting

> ***WARNING!***
> **Always turn your CelliGen BLU off and disconnect the power cord before performing maintenance.**

As with any equipment, difficulties sometimes arise. If you experience a problem with the operation of your CelliGen BLU, consult the following list of symptoms. You may be able to resolve the situation easily and quickly yourself.

If the problem is not listed below, or if the suggested solutions do not work, please call your NBS representative to request a service technician. **Other than the solutions proposed below, do not attempt to fix the equipment yourself.**

Problem	Possible Solution
TEMPERATURE:	
Readout is a negative value (typically –225° C).	• Make sure the temperature probe is connected to the cabinet jack.
The unit will not heat up.	• Make sure the temperature probe is plugged into the vessel thermowell.
AGITATION:	
Agitator does not turn, or turns only slowly.	• The motor drive may not be seated securely on the coupling; check the orientation of the notches. • Make sure the motor is plugged into the cabinet receptacle; TURN OFF MAIN POWER BEFORE CONNECTING THE PLUG.

…continued…

Problem	Possible Solution
DO and pH PROBES:	
DO probe readings are erratic.	• Recalibrate the probe, carefully following instructions in this manual. • Recharge the probe, carefully following instructions in this manual. • Probe may need a new membrane and a refill of electrolyte. • Check for a secure connection. • Replace probe cable or DO probe.
pH probe readings are erratic.	• Recalibrate the probe, carefully following instructions in this manual. • Check for a secure connection.
GASFLOW:	
There is insufficient gas flow.	• Check that the air pressure is within the specified range. • Make sure the control mode for DO and for pH is set to **AUTO** (not **OFF**). • Make sure that the GasFlow loop is ON. • Make sure that the Air loop is in O2 Enrichment mode. • Make sure that the DO cascades are Enabled.
GENERAL:	
Touchscreen is not responding.	• Calibrate touchscreen.

19.2 Product Return

If you should ever need to return your CelliGen BLU to NBS for servicing or any other reason, you will need a product return authorization. Please consult Section 25, *Appendix E: Product Returns*, for instructions.

20 DRAWINGS

Figure 49a: Control Schematics, 120V

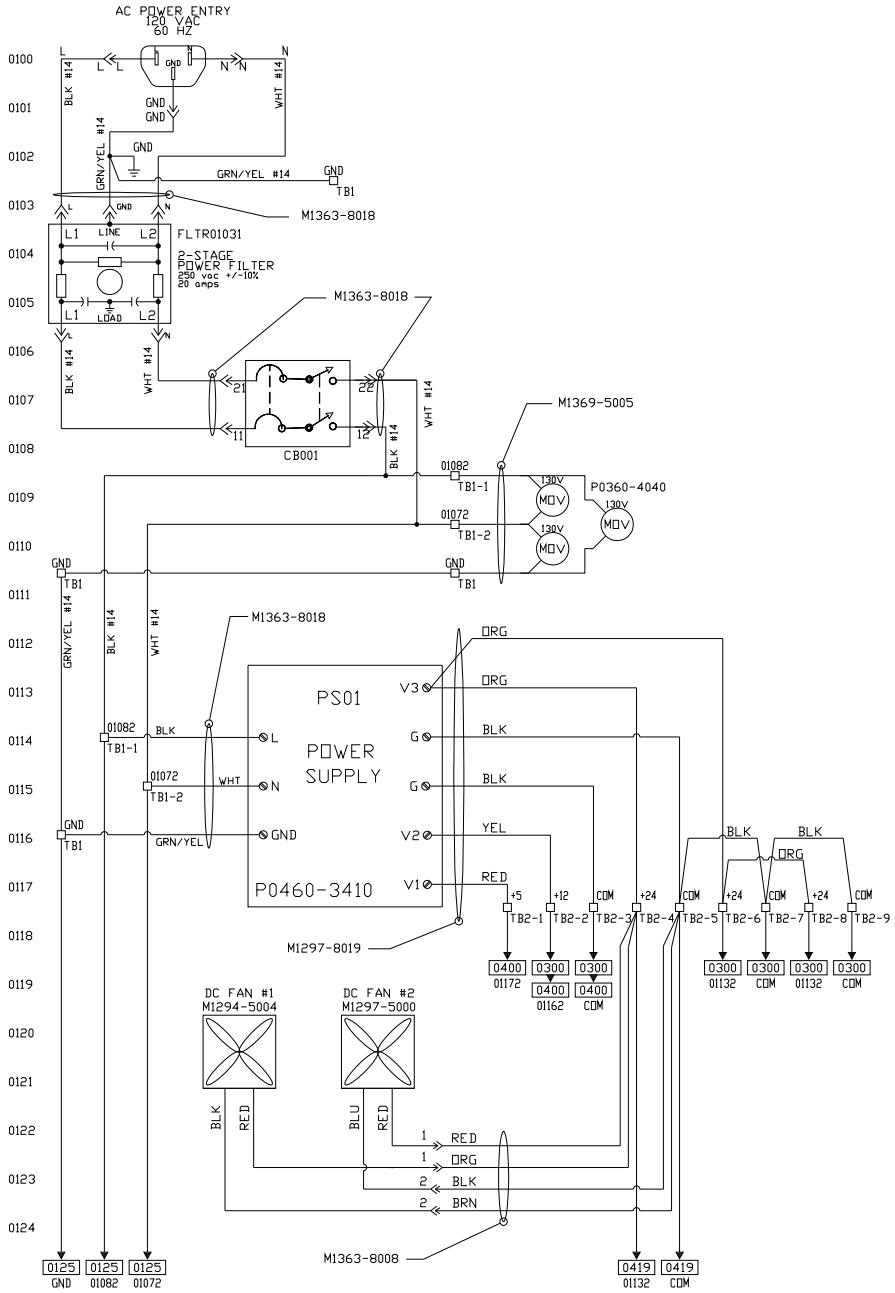


Figure 49b: Control Schematics, 120V

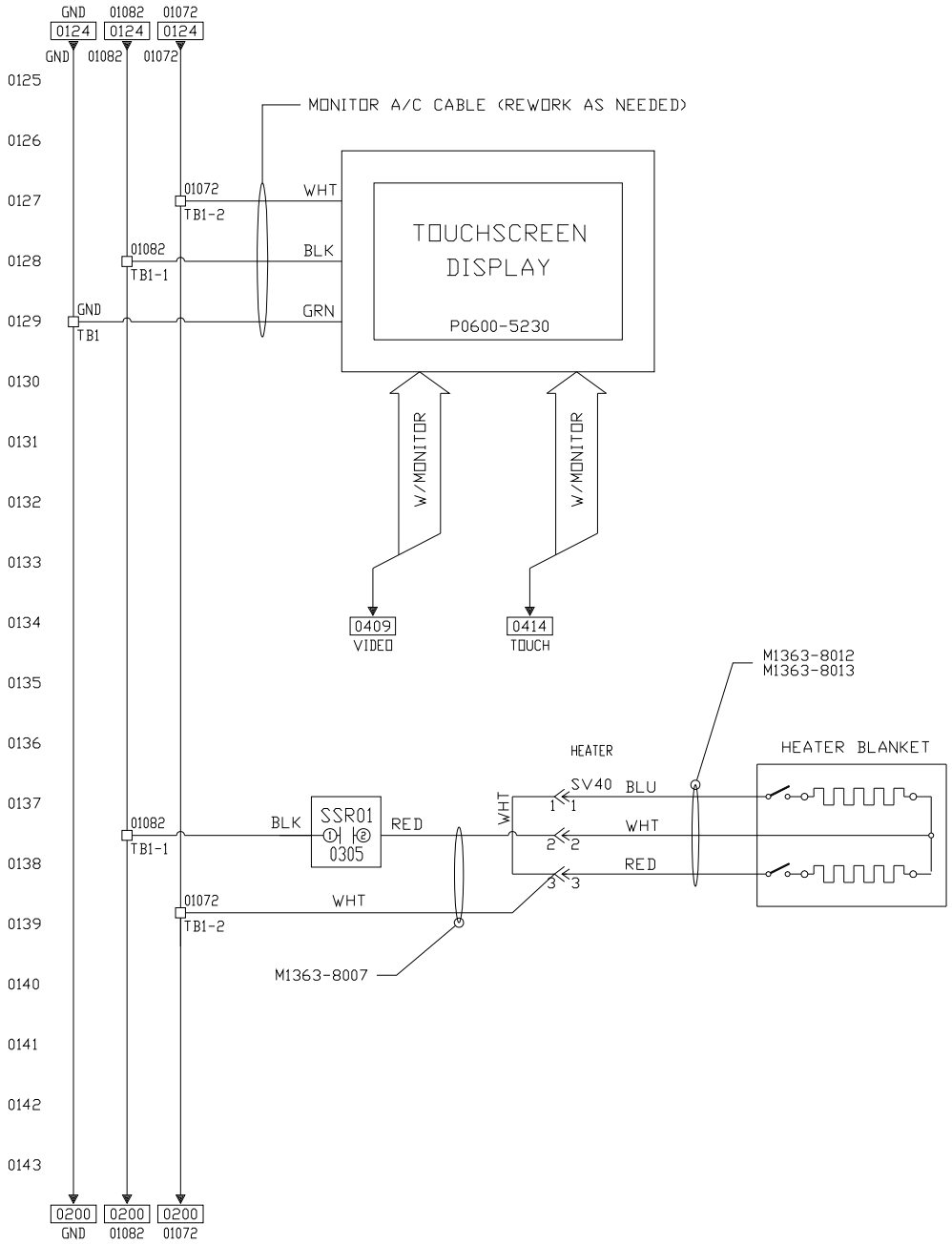


Figure 49c: Control Schematics, 120V

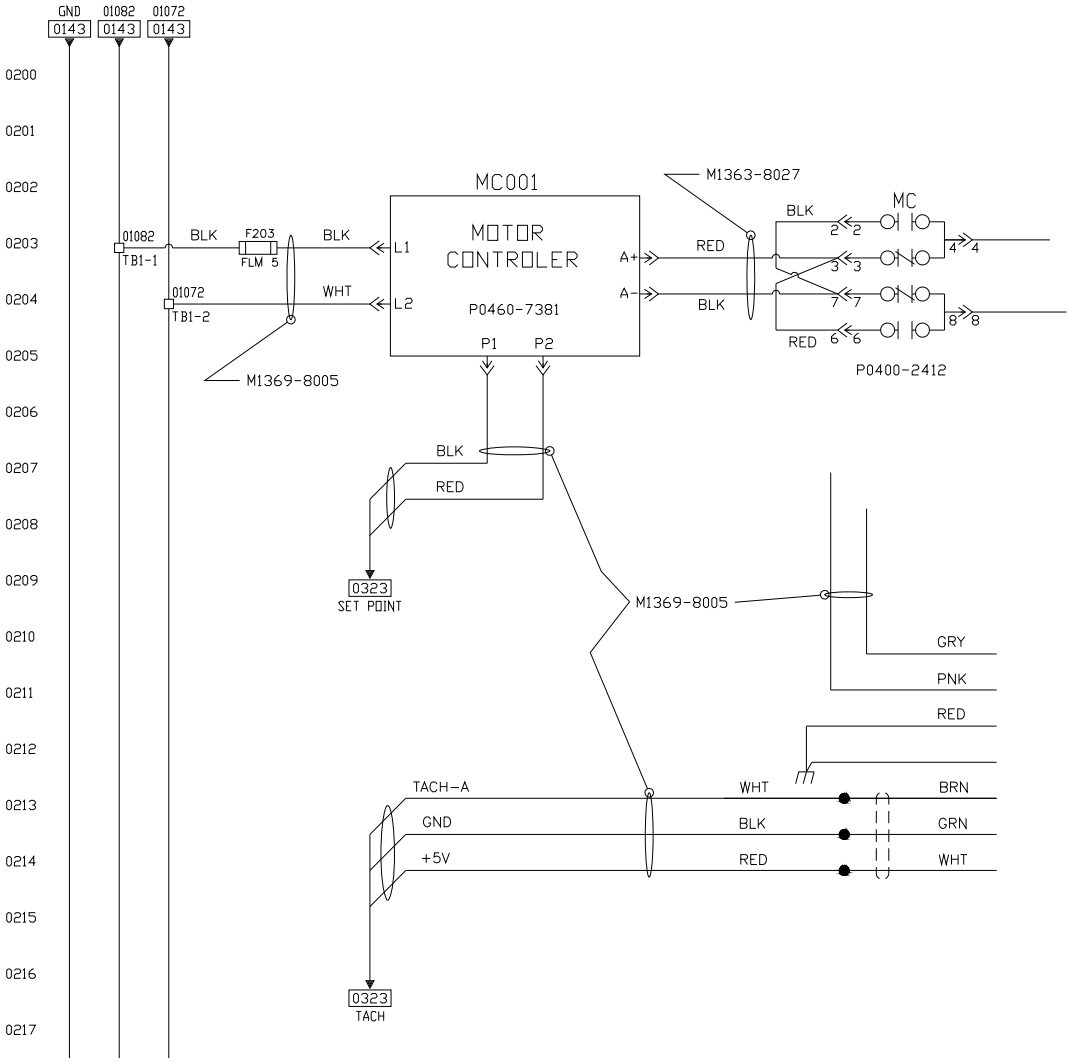


Figure 49d: Control Schematics, 120V

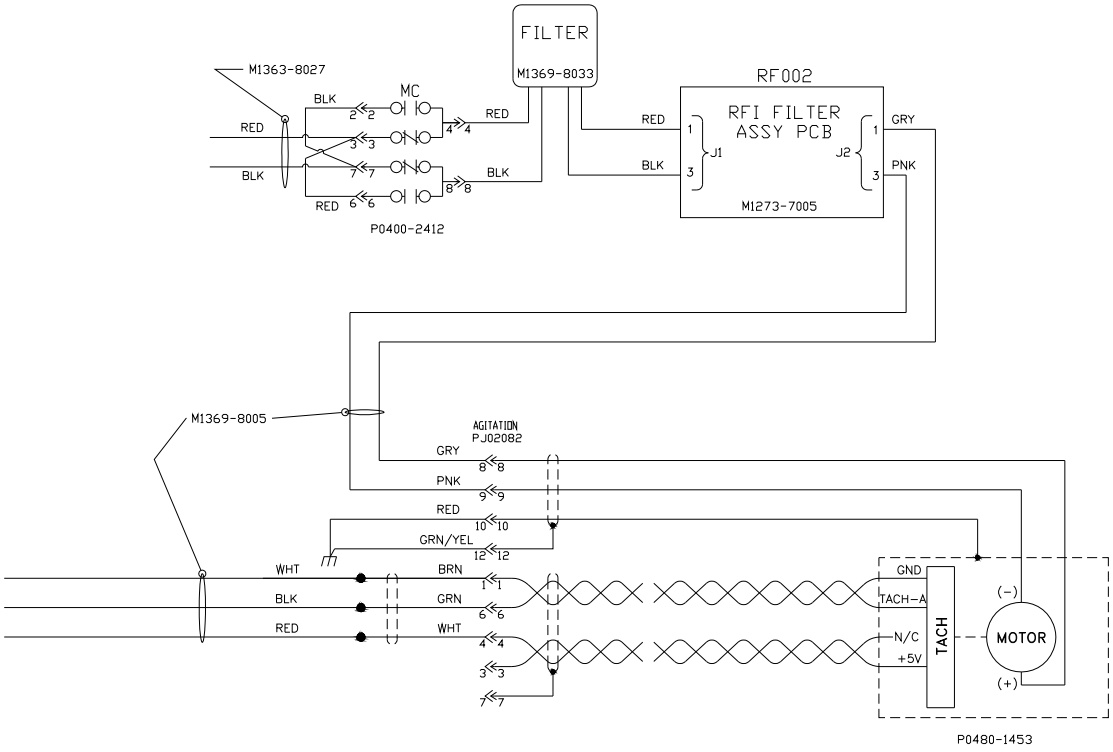


Figure 49e: Control Schematics, 120V

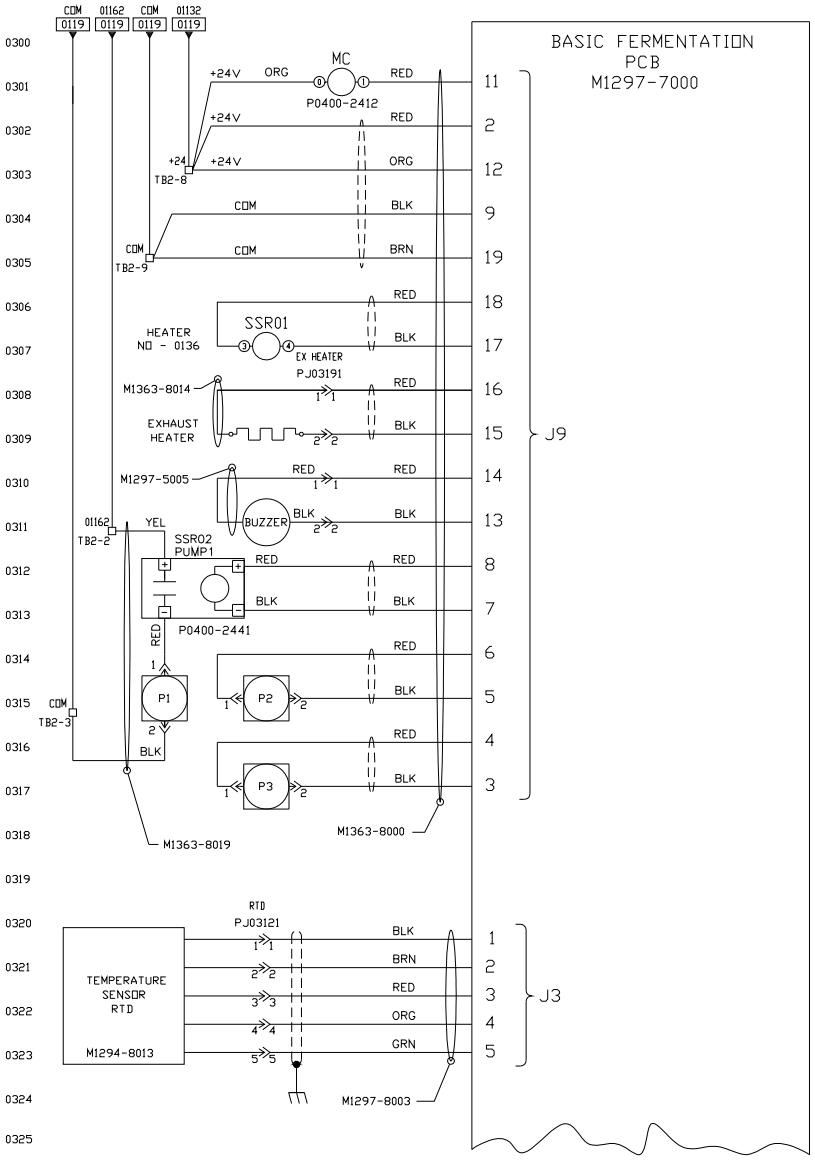


Figure 49f: Control Schematics, 120V

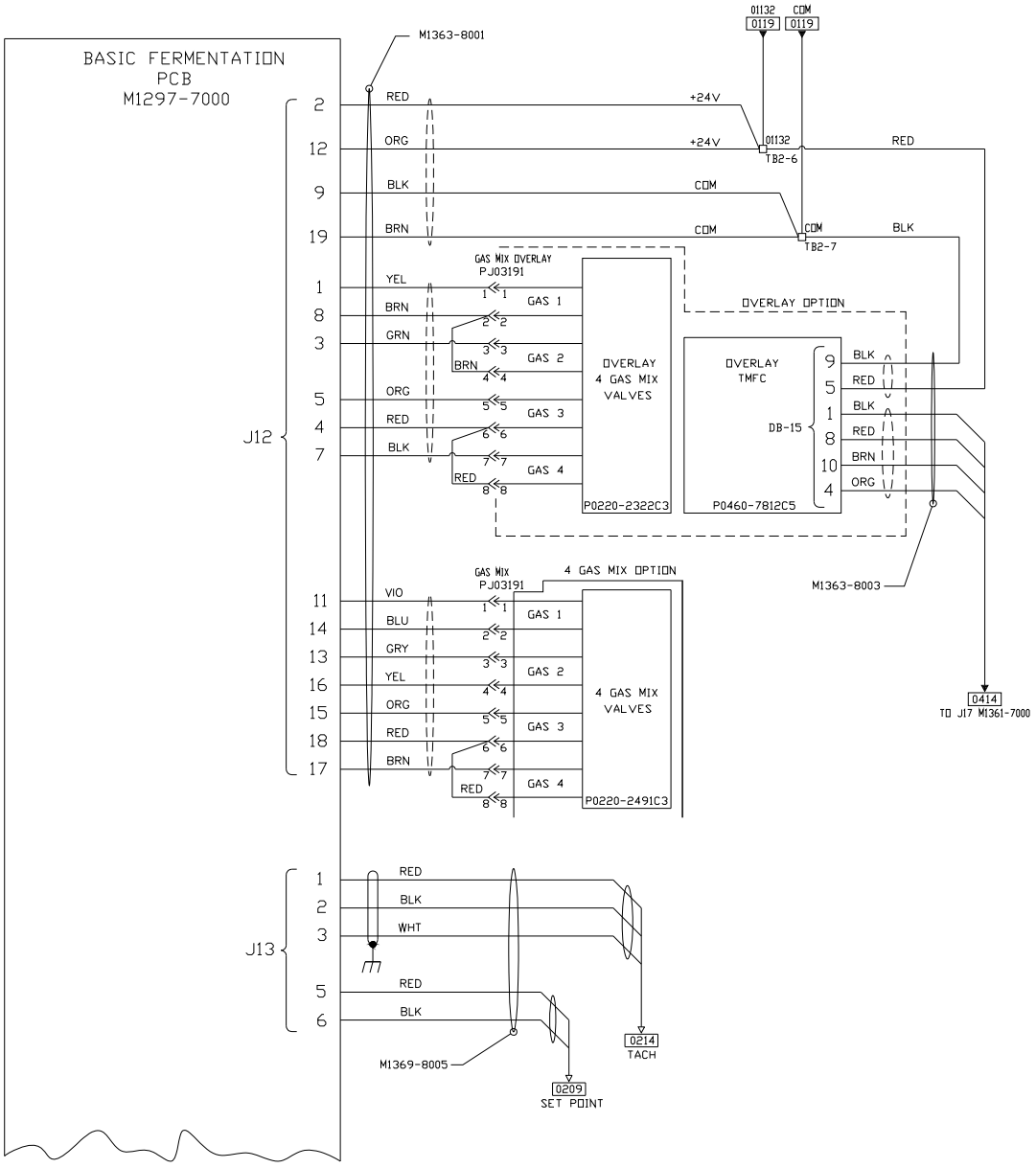


Figure 49g: Control Schematics, 120V

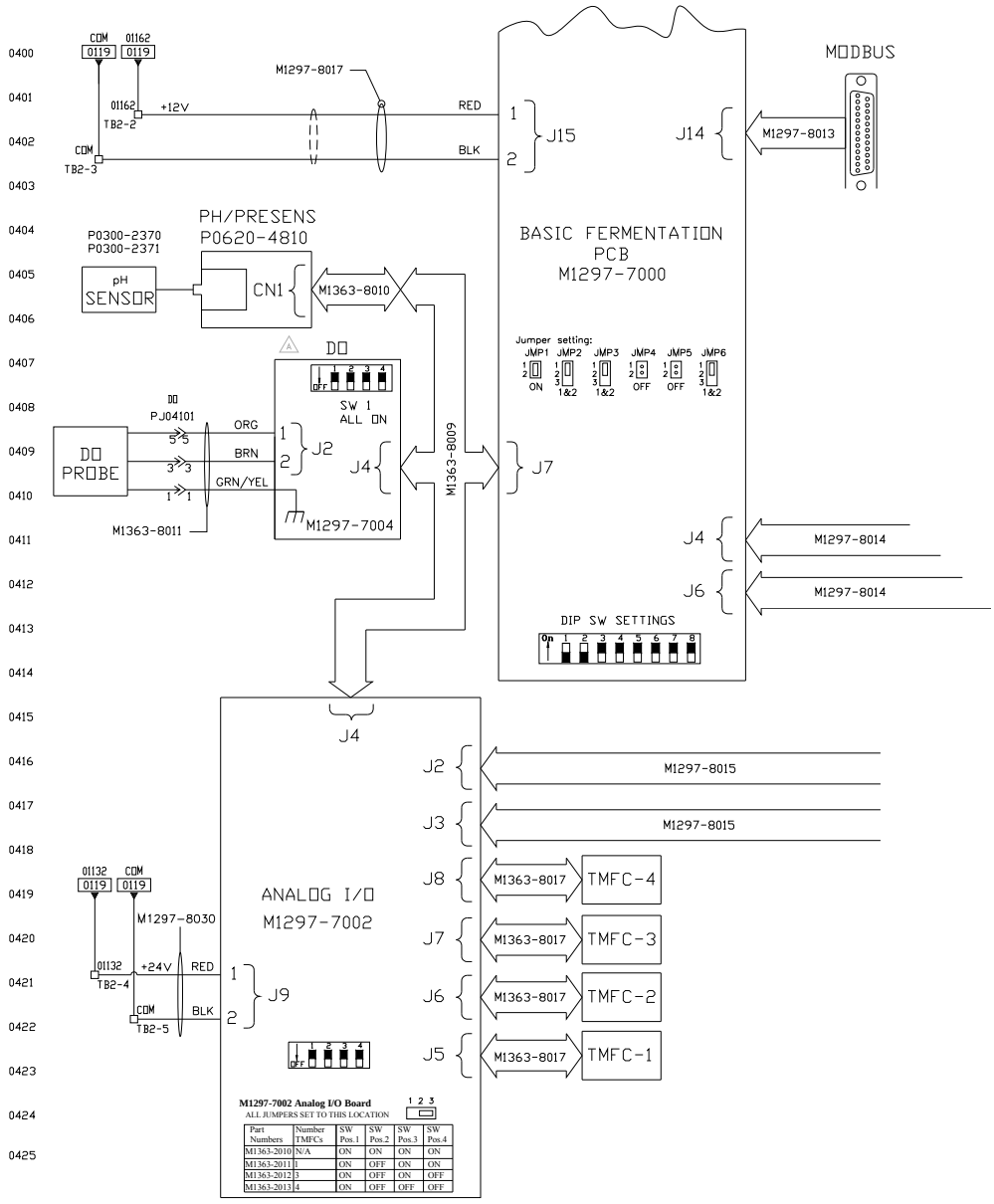


Figure 49h: Control Schematics, 120V

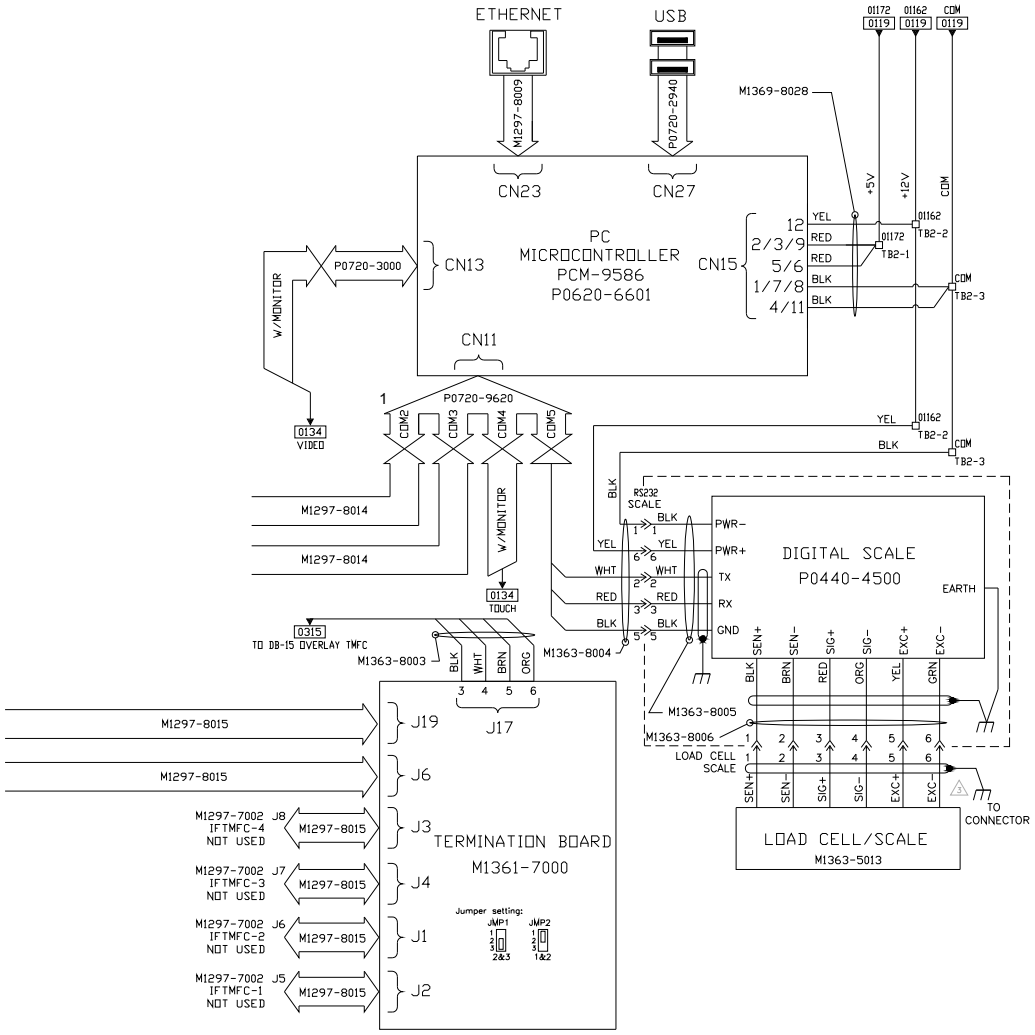


Figure 50a: Control Schematics, 230V

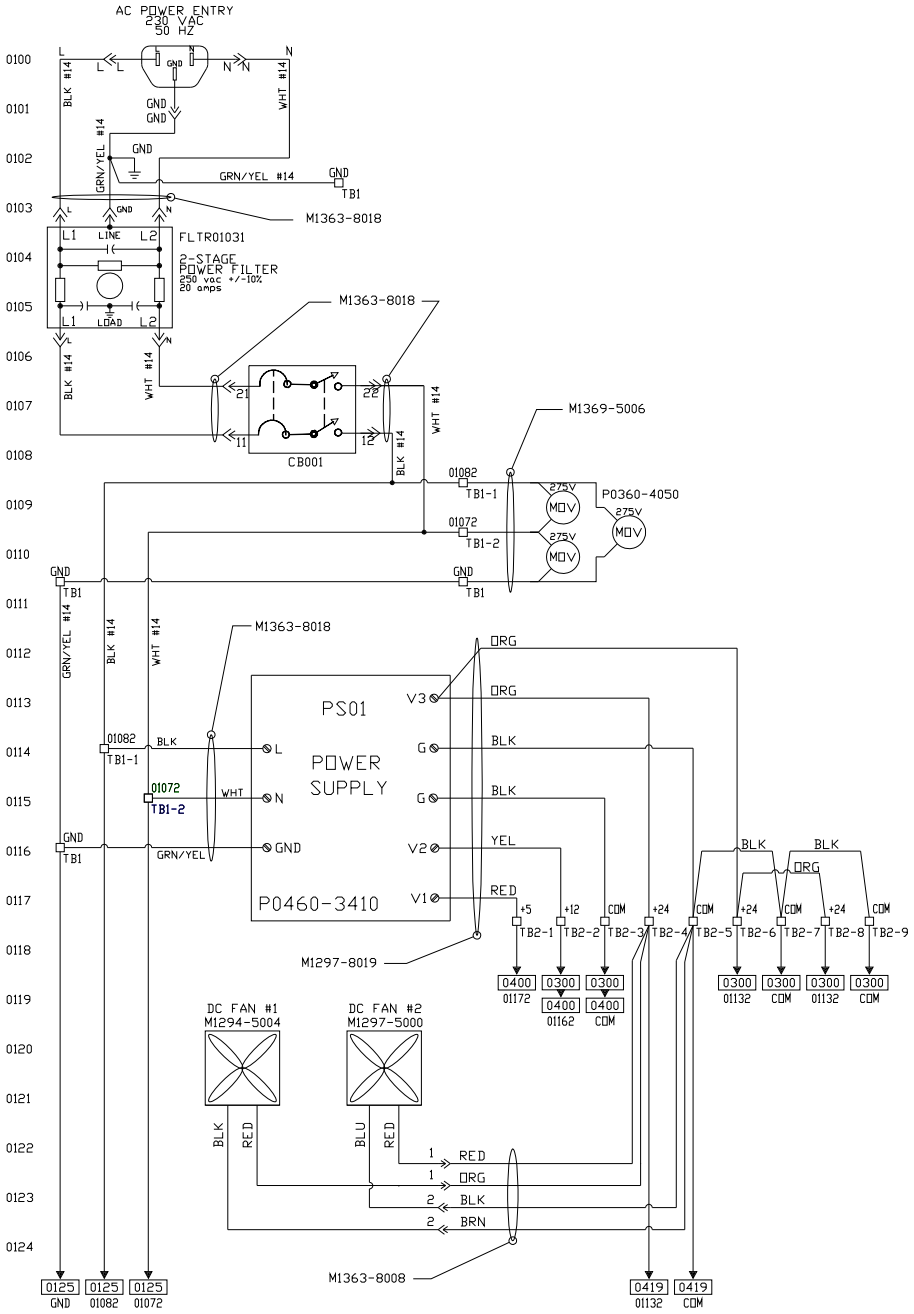


Figure 50b: Control Schematics, 230V

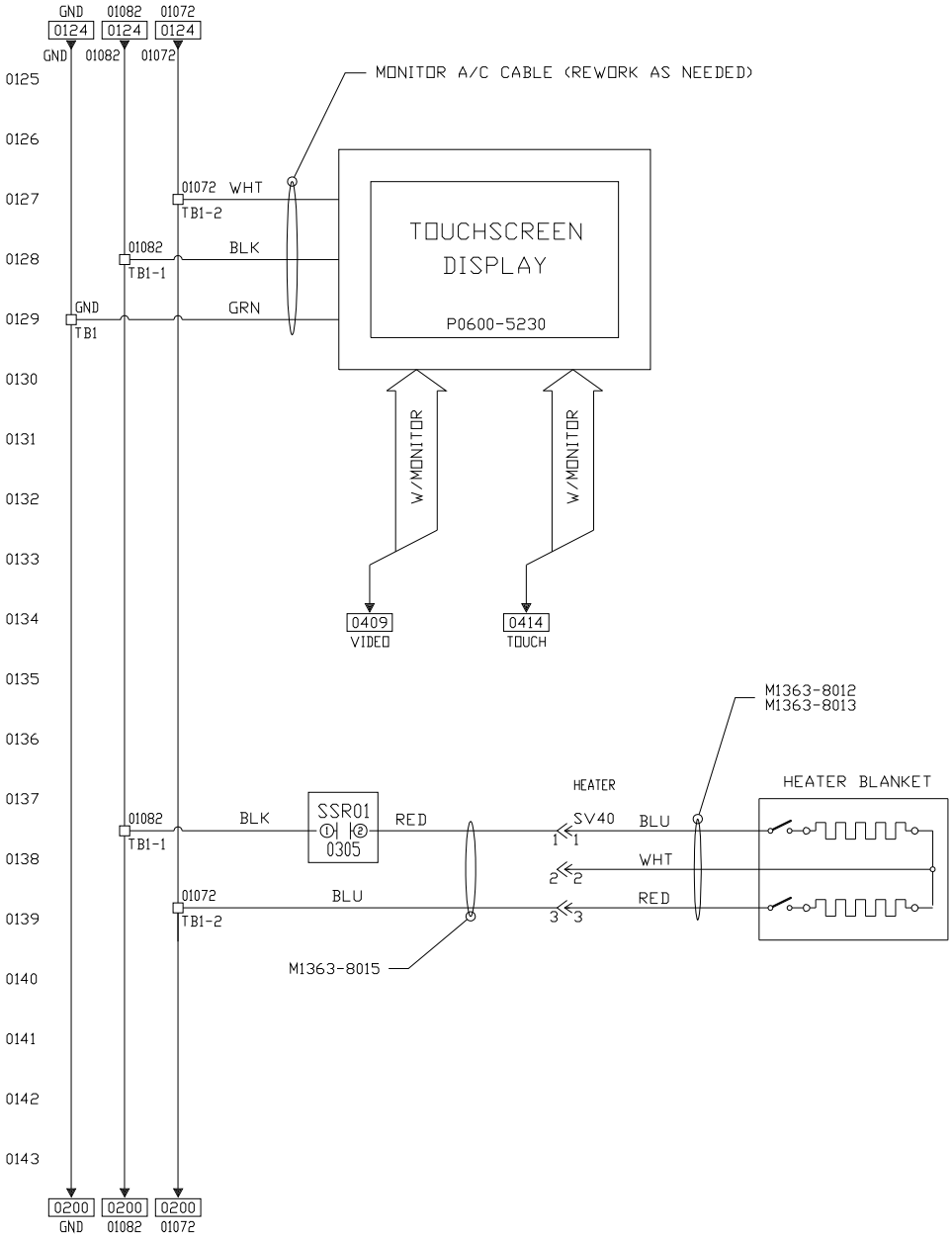


Figure 50c: Control Schematics, 230V

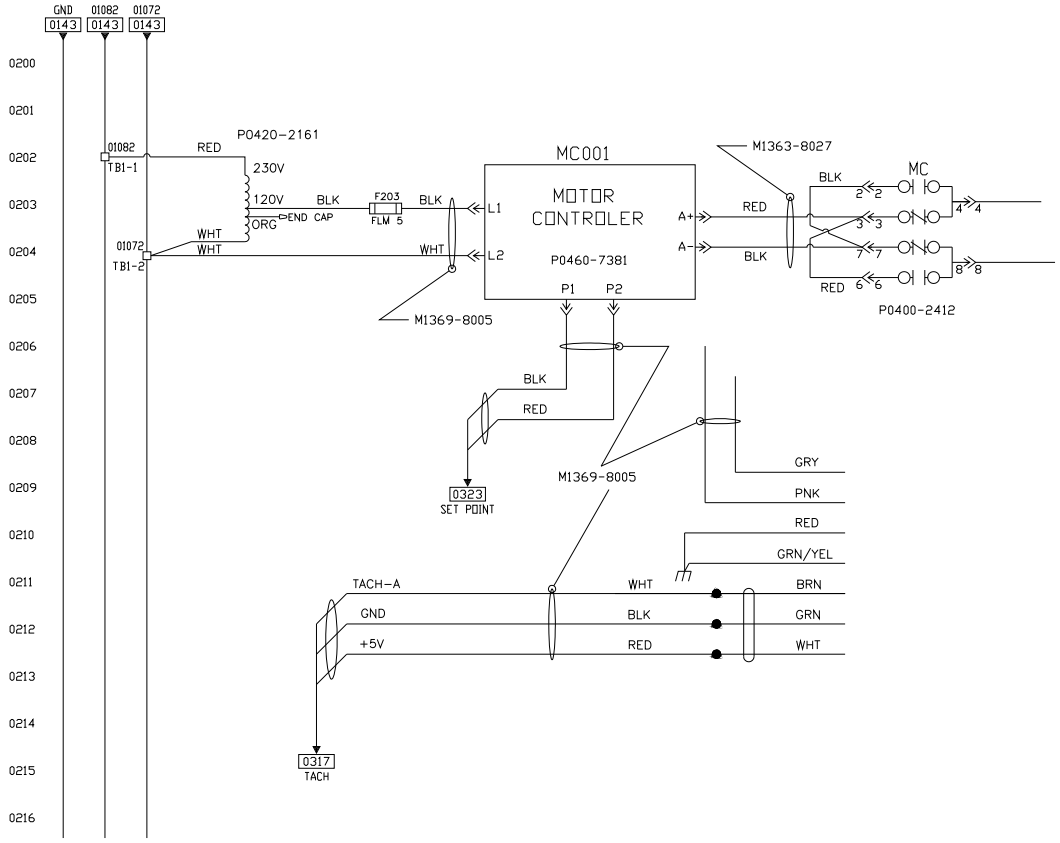


Figure 50d: Control Schematics, 230V

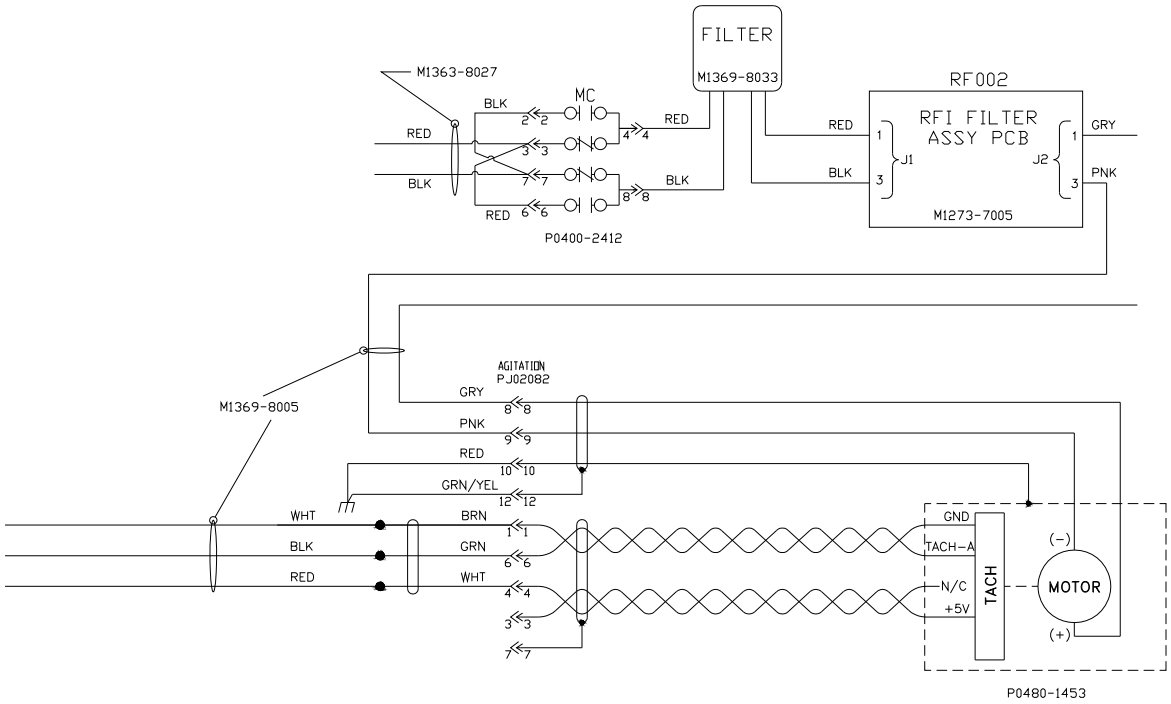


Figure 50e: Control Schematics, 230V

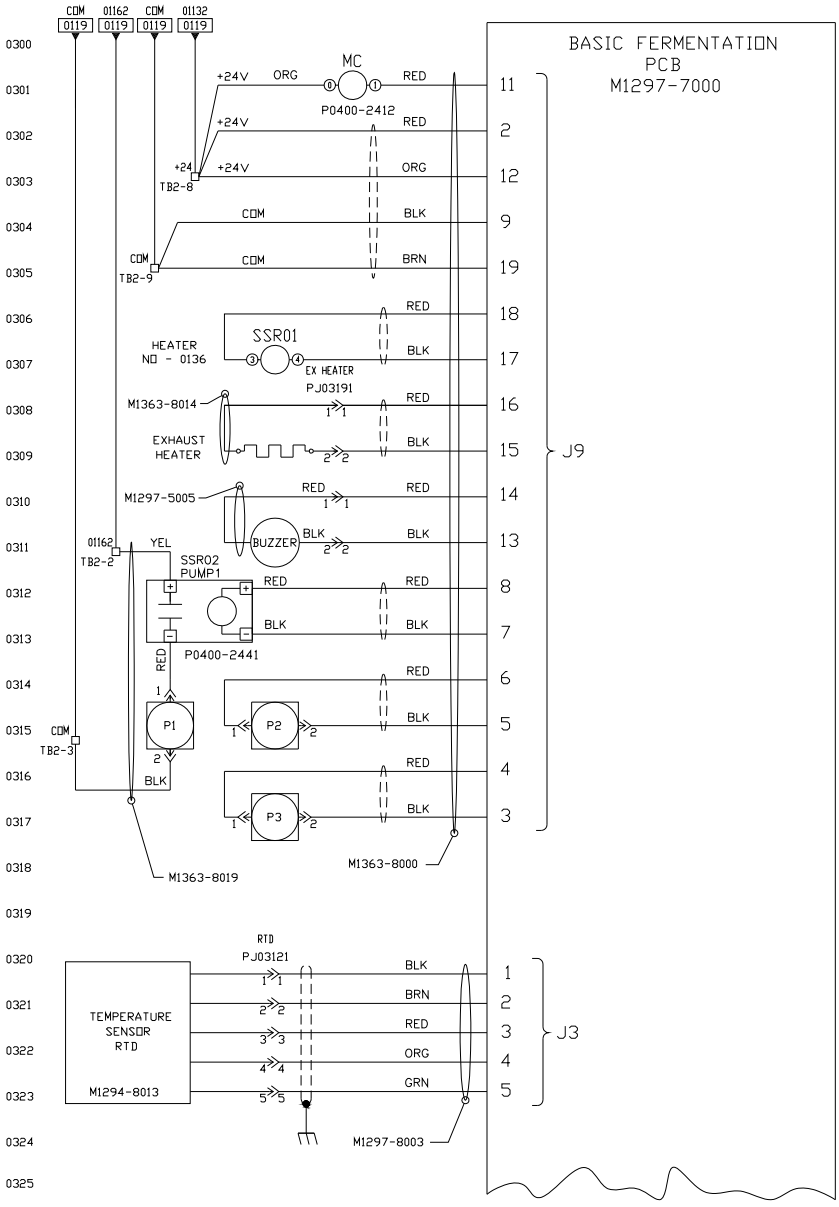


Figure 50f: Control Schematics, 230V

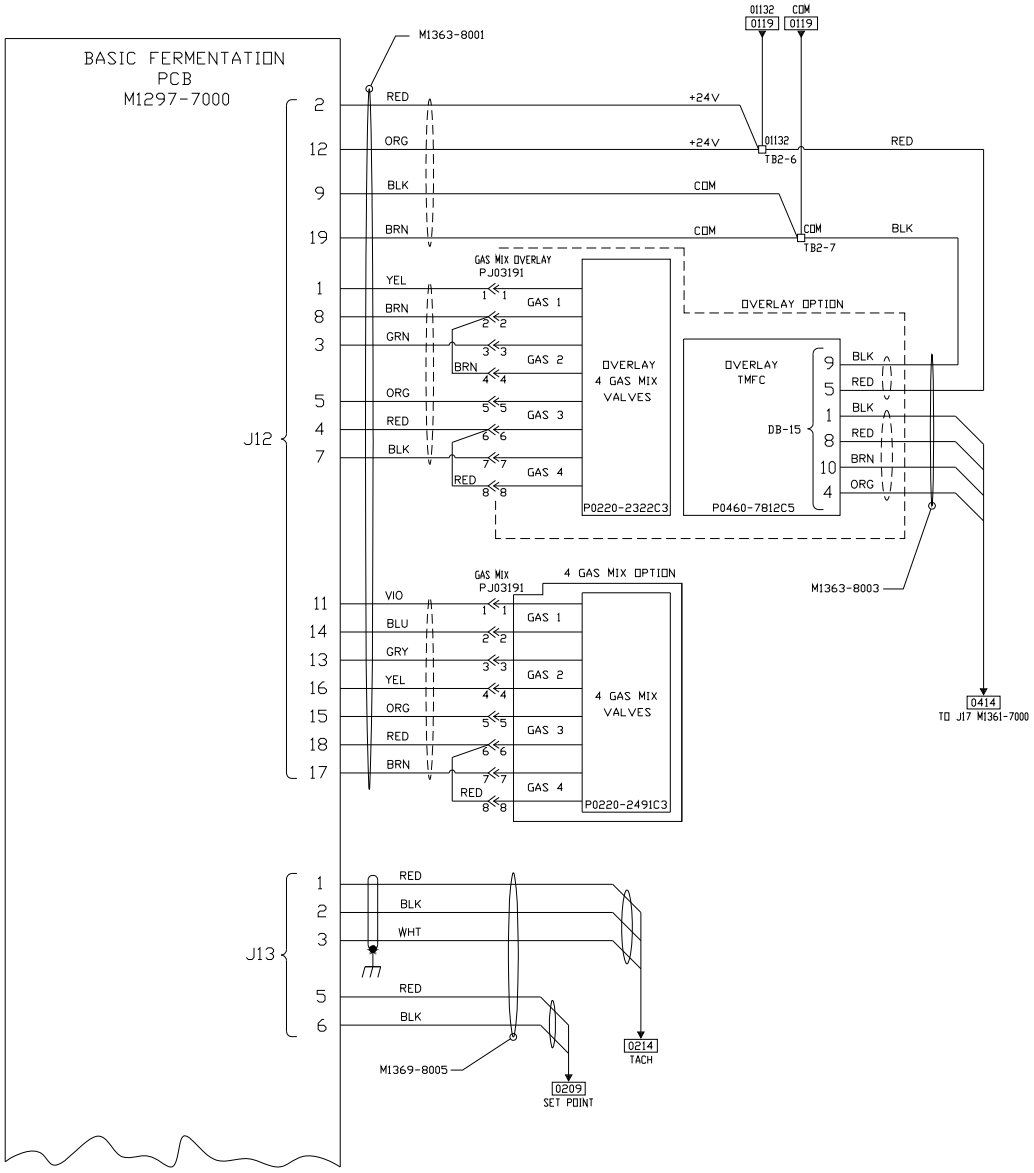


Figure 50g: Control Schematics, 230V

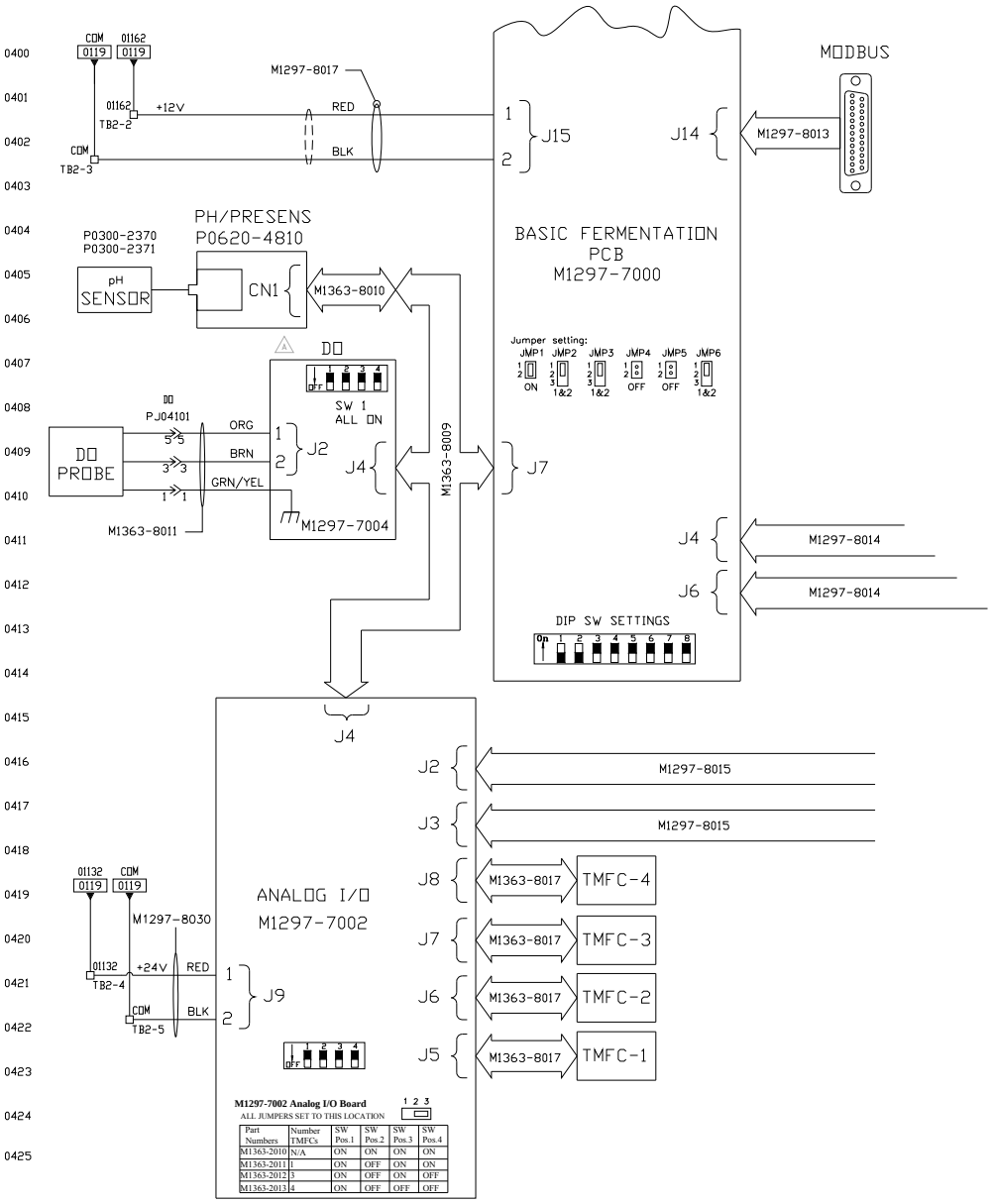
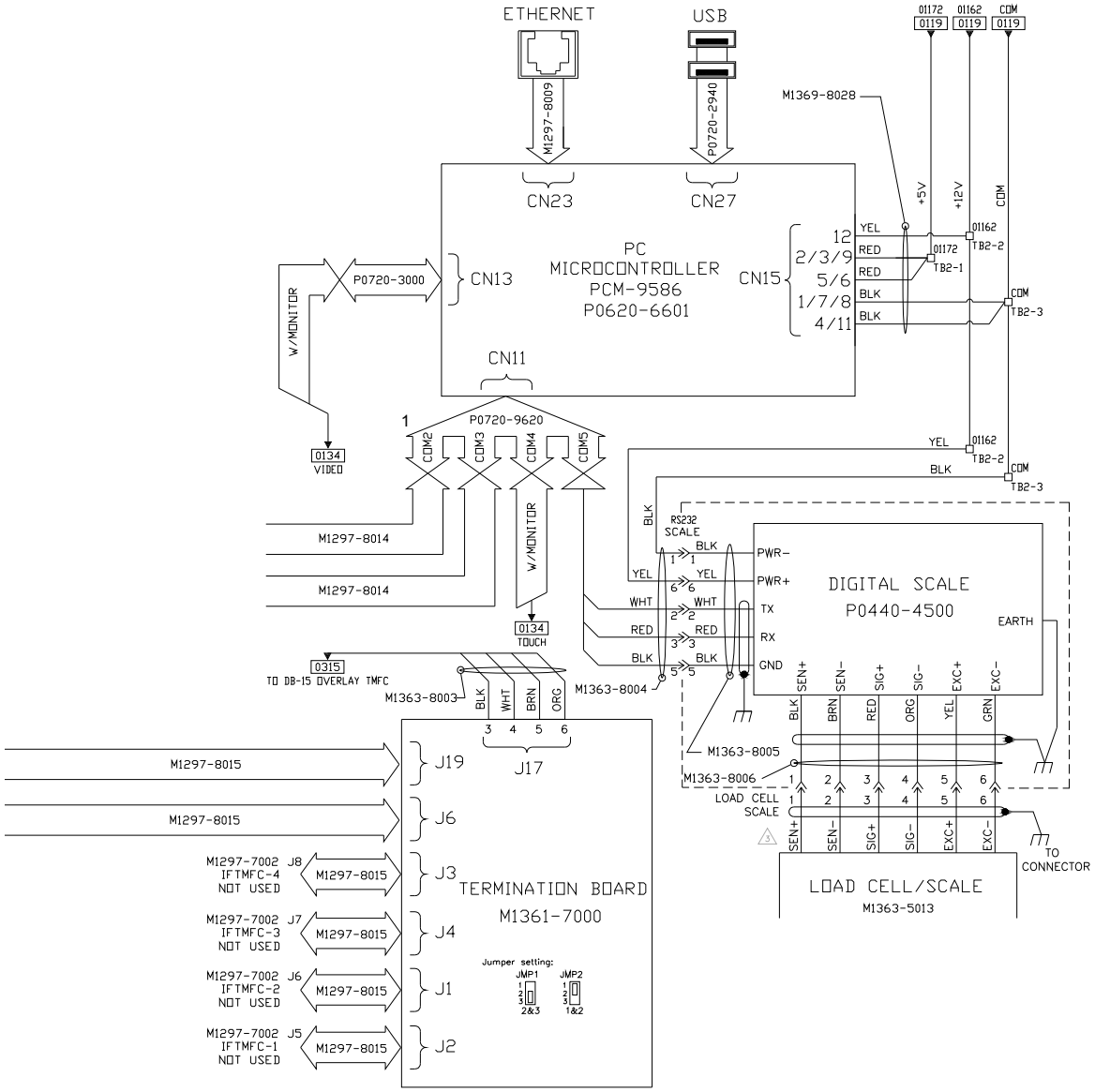


Figure 50h: Control Schematics, 230V



20.1 List of Drawings

Figure	Description	Page
1a	Front & Rear Views of Control Cabinet	10
1b	Touchscreen-to-Control Cabinet Connections	11
2	Control Cabinet Service Connections	12
3	Vessel Assembly	15
4a	Headplate Arrangement (shown w/o tubes & connectors)	16
4b	Headplate Arrangement (shown w/ tubes & connectors)	17
5	Internal Vessel Components	19
5a	pH & DO Headplate Ports (close-up)	20
6	Inputs & Outputs for Ancillary Equipment	23
6a	Bag Hanger	24
7	Touchscreen	28
8	Sample SUMMARY Screen	29
9	Sample SYNOPTIC Screen	32
10	Sample GAUGE Screen	33, 37, 56
11	Add User-Defined Loop Screen	34, 87
12	LoopName Touchpad	34
13	Deleting a Control Loop	35
14	Deleting a Pump Control Loop	36
15	Calibration Screen	37, 45
15a	Probe Data Screen	46
16	Cascade Screen	38, 62
16a	Cascade Screen (detail)	60
17	Trend Screen	39, 77
18	Pumps Screen	39, 57
19	Alarms Screen	40, 88
20a	Controller Setup Screen	41, 93, 94
20b	Recipe Manager Screen	41, 95
20c	System Settings Screen	42, 96
20d	Hardware Setup Screen	42, 97
21	Control Cabinet Rear Panel	43
22	Calibrating DO	48
22a	Setting Zero for Scale/Volume Calibration	49
22b	Setting Span for Scale/Volume Calibration	50
23	Standard Pump Array (pumps open)	51, 82
24	Typical Liquid Addition System	52
25	Setpoint Touchpad	56
26	Pump Assignment Pane	58, 59
27	Sample DO Cascade	63
28	Controller Setup Screen (0 TMFC)	65
29a	Air (1) Gauge with 0 TMFC & 3-Gas Control	66
29b	Air (1) Gauge with 0 TMFC and 4-Gas Control	66
29c	Air (1) Gauge with 0 TMFC & Manual 3-Gas Control	68

...continued...

Figure	Description	Page
29d	Air (1) Gauge with 0 TMFC and Manual 4-Gas Control	68
30	Controller Setup Screen (1 TMFC)	69
31a	Air (1) Gauge Screen with 1 TMFC & 3-Gas Control	70
31b	Air (1) Gauge Screen with 1 TMFC & 4-Gas Control	71
31c	Air (1) Gauge Screen with 1 TMFC & Manual 3-Gas Control	71
31d	Air (1) Gauge Screen with 1 TMFC & Manual 4-Gas Control	72
31e	Sample Cascade Screen with 3- or 4-Gas Mix	72
32	Controller Setup Screen (3 TMFCs)	73
32a	AirFlo (1) Gauge with 3 TMFCs in Manual/Cascade Mode	74
32b	Sample Cascade Screen with 3 TMFCs	74
32c	AirFlo (1) Gauge with 3 TMFCs in 3-Gas/Auto Mode	75
33	Controller Setup Screen (4 TMFCs)	76
33a	AirFlo (1) Gauge with 4 TMFCs in Manual/Cascade Mode	76
33b	Sample Cascade Screen with 4 TMFCs	77
33c	AirFlo (1) Gauge with 4 TMFCs in 4-Gas/Auto Mode	78
33d	AirFlo (1) Gauge with 4 TMFCs in 3-Gas/Auto Mode	78
34	OvlMix Gauge Screen	79
35	Trend Setup Screen	82
36	Trend Graph	83
37	Selecting Zoom Coordinates	84
38	Selecting a Read Line Location	85
39	Setting Pump Setpoint	87
40	Calibrating the Pump Flow Rate	88
41	Pump Period (Sec)	89
42	Variable Speed Pump	90
43	Rear Panel of CelliGen BLU Cabinet	90
44	Sample Loop Alarms Screen (pH)	94
45	Sample Alarms History Screen	96
46	Security Settings Screen	102
47	Security Keypad	103
48	User Button	103
49a-h	Control Schematics, 120V	113-120
50a-h	Control Schematics, 230V	121-128
51	Mounting Base Pump for Stacking	132
52	Stacking the Pumps	133
53	Stacked Pumps	133
A	Cell growth over the 7-day run [CHO cells]	143
B	Cell viability over the 7-day run [CHO cells]	144
C	Glucose consumption/lactate production 10-day run [CHO cells]	144

20.2 List of Tables

Table	Description	Page
1	Service Connections	13
2	Headplate Tubing & Connections	18
3	SUMMARY Screen Features	30
4	Modbus Com Port Pin Designation	44
5	Gas Overlay Operating Modes	80
6	Trend Graph Buttons	83
7	Pump Control Modes	87
8	Flow Rate per Tubing Size	88
9	Alarms Screen Features	93

21 APPENDIX A: STACKABLE PUMPS

A novel optional feature of the CelliGen BLU pumps is that you can add another identical pump onto the front of one of the pumps in the array. The pump that serves as the base, however, must be specially ordered to have no lower front cover and an exposed screw face on which to mount the second pump.

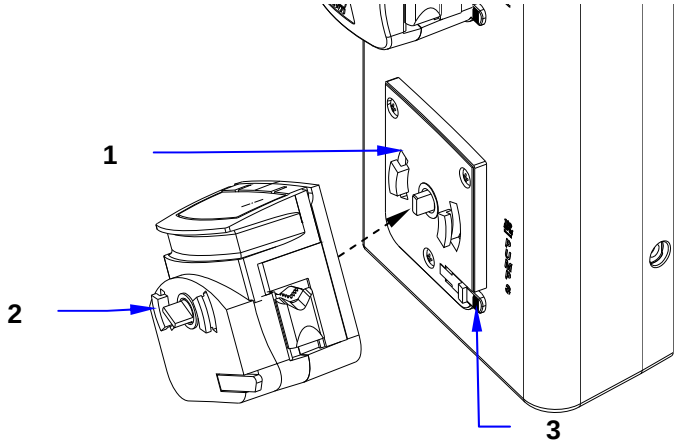
The stacked pump will be twinned to—in other words, controlled in exactly the same way, at the same time, as—its base pump.

To stack a pump (*with reference to Figures 51 & 52*):

1. Remove the standard pump that you wish to stack by pressing the pump release lever, turning the pump to the left until it clicks, then pulling it away from its baseplate mating surface (*see baseplate in Figure 51*).

2. Carefully align the center hole on the back of the special base pump with the "nose" of the baseplate screw face, also aligning the curved slots (like parentheses on either side of the hole) with their male counterparts on the baseplate screw face—you will need to tilt the pump a little to the left to catch all three mating surfaces (*see Figure 51*).

Figure 51: Mounting Base Pump for Stacking

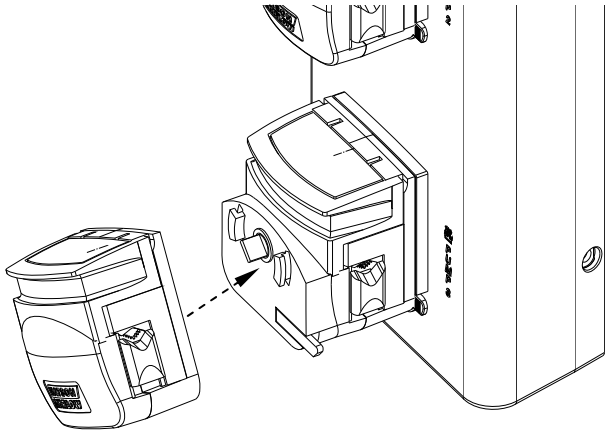


1	Baseplate screw face
2	Exposed screw face of base pump
3	Pump release lever

3. Once all three mating surfaces are aligned, push the pump back against the baseplate, turning it until it is straight upright and snaps into place.

4. Now add the standard pump in the same way to the screwface of the base pump *(see Figure 52*).

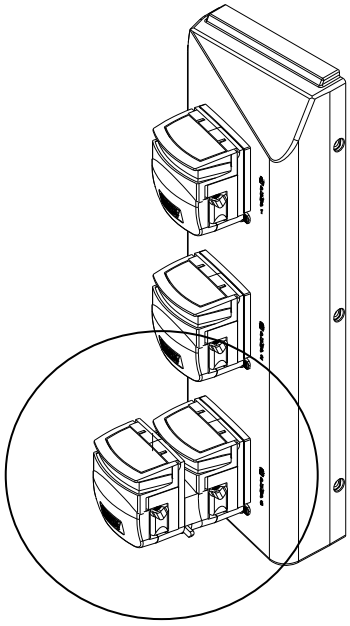
Figure 52: Stacking the Pumps



5. When you have stacked pumps, load the tubing one pump at a time, starting with the base pump. If you open both at the same time, the back pump channel will be blocked by the front pump's cover.

Figure 53 shows two pumps stacked:

Figure 53: Stacked Pumps



22 APPENDIX B: SOME GENERAL CONCEPTS

NOTE:
In this section, all discussions of P-I-D control are to explain the theory on which it is based. This product uses only P (proportional) & I (integral) control, not D (derivative).

22.1 What is a Controller?

The local process controller is a multi-loop controller, which means it can control several process parameters simultaneously. It compares process values with setpoints and creates independent control signals for each controlled parameter. The control signals are used to drive appropriate actuators that maintain the various parameters at their setpoints.

Using temperature as an example, the controller compares the output of a temperature sensor to the user-entered temperature setpoint, and generates a signal to activate either a heater or a cooler to maintain vessel temperature at the temperature setpoint. The controller provides the logic that generates appropriate drive signals to various actuators so that process parameters remain at their setpoints.

22.2 What is a Control Loop?

A control loop is the basic element of automatic process control. Three components comprise one control loop: a sensor, a controller, and an actuator. Based on information from a sensor, the controller generates an actuator control signal that maintains a parameter at its setpoint. Control will fail if any element in the control loop fails.

22.3 What is Probe Calibration?

In bioprocess control, *calibration* generally refers to establishing a correspondence between a probe's output and the actual value of whatever that probe senses. For example, pH probes are often calibrated with pH 7.0 and pH 4.0 buffers to establish a "zero" (pH 7.0) and a "span" (pH 4.0). Other buffers can be used, but the principle is always the same. For any probe calibration, two values—a zero and a span—are required for the controller to correctly translate inputs from that probe. DO and pH probes are routinely calibrated before each use. Most other probes need be calibrated only infrequently.

22.4 What are P-I-D Constants?

The mathematics of **P-I-D** control is familiar to most control and process engineers.

In **P-I-D** mode, the controller creates a control signal that is based upon setpoint and input from a sensor. The magnitude of the control signal is determined by a mathematical formula that can include proportional ("P"), integral ("I") and derivative ("D") terms. The **P**, **I** and **D** constants are three numbers that determine the relative sizes of the proportional, integral and derivative terms, respectively. To use a temporal analogy, the **P** or proportional part of the control signal reflects present deviations between setpoint and process value. The **I** or integral component reflects past deviations, and the **D** or derivative term anticipates future values of the error.

Generally, with noisy or slow-responding sensors, such as dissolved oxygen and pH probes, the **D** constant should be set to zero. If the constants for a loop are too large, that loop will oscillate, displaying extreme swings in actuator output. If, for example, agitation changes suddenly and frequently between minimum and maximum RPM, one should suspect incorrect **P**, **I** and **D** values for the agitation control loop. This condition can easily be mistaken for a defective component when it actually results from incorrect settings.

If the constants are too small, control response will be slow, and setpoints may never be reached. Again, this can be mistaken for defective components. **P-I-D** constants are usually established by methodical trial and error.

22.5 What is P-I-D Tuning?

Tuning consists of establishing controller settings (the proportional, integral, and derivative constants) such that the controller provides proper control. If the **P-I-D** constants are incorrect, the control signal may be too weak for the parameter to ever reach setpoint or, at the other extreme, the controller may respond excessively to small errors, causing the actuator to oscillate between high and low values. Usable **P-I-D** constants must be determined for each **P-I-D** loop. The process is largely one of calculated trial and error.

All loops that are configured with the **P-I-D** control mode must be tuned. When delivered as part of an NBS system, **P-I-D** loops will have been tuned at the factory to work correctly with the NBS-controlled instruments. For other applications, the user is responsible for **P-I-D** tuning.

Tuning can be a complex task for those unfamiliar with the process, which is why a trained engineer or technician normally performs this task. A number of textbooks[1] that explain the theory and describe the process could be useful for the mathematically-inclined novice. The Ziegler-Nichols method, described in the footnoted reference, is used at our production facilities.

The following suggestions are intended for novices. Be sure to refer to a textbook, and consider utilizing the services of a technician.

- Allow sufficient time for the task. Tuning is an iterative process. It consists of configuring a loop with trial **P**, **I** and **D** values, evaluating loop response, then readjusting the constants. The process is repeated until the loop responds fully and without oscillation.

- One usually begins with a trial **P**, setting **I** and **D** to zero. After **P** is established, a similar iterative process establishes **I**.

- Most bioreactor probes respond too slowly or are too noisy to utilize the **D** term to advantage. In most cases, **D** should remain at zero. Agitation is sometimes an exception.

- The magnitude of the control signal depends on the **P**, **I** and **D** constants. It also depends inversely on a *Normalizing Constant.*

[1] For example, Chinks, F.G., Process Control Systems: Application, Design, and Tuning, McGraw-Hill (1988), New York, Auckland, Bogota, London, Toronto, Sydney, Tokyo, Montreal.

22.6 What Do the Constants Mean?

The control signal, S_N, for a loop that has been N seconds in **AUTO** mode is expressed mathematically as:

$$\mathbf{S_N = P(e_N/k) + \Sigma(I/60)(e_n/k) + D[(e_N - e_{N-1})/k]}$$

Where:

P, **I**, and **D** are, respectively, the proportional, integral and derivative constants

e is the loop setpoint minus the process value, or error

k is a normalizing constant for the loop

The controller reevaluates S_N every second. **I** is divided by 60, so any value entered by the user should be in reciprocal minutes.

The normalizing constant k can be set to any non-zero value, but is usually set to the full-scale reading of the loop. For example, if the range of expected temperatures is 0 to 125, setting k to 125 results in a **P** term value of **P** when the error is at a maximum, i.e.:

$$\mathbf{P(e_N/k) = P(125/125) = P}$$

Similarly, with a full-scale error, the **I** term (after 1 minute) and the **D** term will be **I** and **D** respectively.

23 APPENDIX C: CORROSION RESISTANCE

Websites such as www.outokumpu.com provide up-to-date information about the 316 type stainless steel used in your CelliGen BLU vessels.

24 APPENDIX D: PROTOCOL FOR GROWING CHO CELLS

24.1 Abstract

The study presents a typical protocol for the setup and operation of New Brunswick Scientific's new CelliGen® BLU single-use, stirred-tank bioreactor, a versatile new benchtop system for the culture of a wide range of mammalian cells. This bioreactor has been designed to provide research and production facilities with a single-use vessel which combines the benefits of both traditional stirred-tank design and single-use technology, capable of seamless process scale-up.

The system can be operated in batch, fed-batch or continuous modes. A procedure for culturing Chinese Hamster Ovarian (CHO) cells in a 5.0 Liter (L) vessel, using CD CHO serum-free medium in a batch culture is described.

24.2 Introduction

Historically, stirred-tank fermentors and bioreactors have been the trusted design for culturing all types of submerged cultures including suspension and anchorage-dependent mammalian cells, insect, yeast, plant and microbial cultures. The tried and tested tank design offers scalability and proven reproducibility which is pivotal for cost-saving process development and productivity. In the last decade, there has been an increasing acceptance and use of single-use technologies, due to their convenient operation and low start-up cost. Single-use systems eliminate the need for cleaning and sterilization, reduce validation requirements, provide rapid turn-around between runs, and significantly reduce the risk of cross contamination and microbial contamination because the culture vessel is only used once and then discarded.

Although single-use, stirred-tank systems in the 75 – 2000 L scale have been on the market for some time, as have small-scale single-use bags that are gently rocked rather than stirred, until now there has been no single-use stirred-tank system for small-scale work. The new CelliGen BLU fills that void, offering a proven stirred-tank design as well as the benefits of single-use technology in a benchtop system.

24.3 Materials and Methods

24.3.1 Single-Use Vessel

CelliGen BLU single-use vessels are offered in 5.0 and 14.0 L total volume capacities. The vessels are delivered pre-assembled with pitched-blade impeller, porous microsparge, and all the necessary tubing, filters, and connectors; and come sterilized, ready for use right out of the package. All components in product contact are made of materials that meet USP Class VI standards and have been tested for leachables and extractables, making these vessels appropriate for cGMP environments. In this protocol, we describe use of a CelliGen BLU with 5.0 L vessel.

24.3.2 Controller

CelliGen BLU's compact, non-disposable control station is designed to provide advanced process management and monitoring capability, ranging from three fixed-speed pumps for additions and harvesting, to a powerful controller with 15" industrial color touchscreen monitor. Multiple options, including gas flow control, a weight scale, validation packages and more, enable customization to your needs.

The control station used in this protocol was configured with one 2 – 100 cubic centimeters per minute (ccm) Thermal Mass Flow Controller (TMFC) for direct sparging of gases and an integrated gas overlay with 0.1 - 3.0 Standard Liters Per Minute (SLPM) flow rate also regulated by a TMFC. Both the gas flow and gas overlay are capable of 4-gas mixing for automatic pH and Dissolved Oxygen (DO) control. Pumps, temperature control, agitation, as well as all of the other process loops, were controlled and monitored through the powerful Reactor Process Controller (RPC) firmware installed in the controller. DO was monitored using a non-invasive reusable polarographic DO probe; and pH was monitored using a non-invasive optical pH probe and fluorescence sensor.

24.3.3 Inoculum Preparation

One 2.5 mL vial of CHO cells was thawed and used to inoculate a 125 mL shake flask which contained 25 mL of serum-free CD CHO medium (Invitrogen 10743-029) which was pre-warmed to 37.0°C.The flask was placed on an orbital biological shaker (NBS Innova® 2000) placed inside a CO_2 incubator (NBS Galaxy® 170 R), and set at 120 rpm.

The CO_2 incubator was programmed to provide a gas mix of 5% CO_2 and 95% air, at 37.0°C. On day 4, when the viable cell density reached $1.5x10^6$ cells/mL, the cells were transferred into a 500 mL shake flask which contained 100 mL of freshly made, pre-warmed medium and allowed to incubate for 3 additional days at the same conditions as earlier. The cells were then transferred to two 1 L shake flasks, each containing 250 mL of the freshly made medium. The inoculum was grown in the shake flasks until cell density reached 2.0 - 3.0 x 10^5cells/mL, with greater than 90% cell viability, sufficient for the bioreactor inoculation.

24.3.4 Bioreactor Set-Up & Inoculation

One day before the cells reached inoculation density, the growth medium was warmed to 37°C and the DO probe was polarized. For this study, 3.0 L of sterile CD CHO serum-free medium was prepared by pre-warming at 37°C for 24 hours in a CO_2 incubator. During this time, the DO probe was connected to the controller for at least 6 hours to enable polarization, as per the manufacturer's recommendation. Once the medium was warmed and the inoculum grown to sufficient starting density, the CelliGen BLU bioreactor vessel was removed from its sterile packaging and the heat blanket supplied with the unit was wrapped around the outside of the vessel. Next, the vessel containing the cell culture medium was connected to one of the bioreactor vessel's inlet lines using a tube welder. (A tube welder is offered as an optional accessory to the CelliGen BLU. A pre-sterilized medium filter with an attached quick connect or Luer connection can also be used if a tube welder is not available).

Since this was a batch process, all of the medium was pumped into the bioreactor vessel. All additional connections to the controller including sparge, overlay, RTD, pH, and agitation were also made. pH and DO were calibrated through the touchscreen controller, and all process setpoints were entered on the touchscreen using the Control Setpoint values shown below. Once the parameters were at their setpoints, the inoculum flasks were connected to the addition line in a sterile manner using a tube welder and contents were pumped into the bioreactor vessel.

24.3.5 Operational Parameters

Cultivation of animal cells in an environment optimal for manufacture of desired end products require monitoring and control of a substantial number of physical and chemical parameters. Physical parameters include temperature, fluid flow (gas flow and liquid flow) rates and agitation rates. Chemical parameters include the dissolved oxygen (DO) concentration and pH.

Control Setpoints:

Temperature . . . . . .37°C
pH 7.0
DO 40%
Agitation . . . . . 80 rpm

pH Control:

pH control was set to Auto mode, which automatically adds base solution or CO_2 gas to the system based on culture demands.

Deadband……..0.10
PID values……..……..Factory-set default values
Base Solution..……..Sodium bicarbonate, 7.5% solution
Transfer tubing…….Narrow bore silicone tubing with Luer-connection (1⁄18-inch ID & ¼-inch OD)
Vessel inlet……1/8-inch inlet tubing in the vessel headplate

Dissolved Oxygen Control:

DO control was set to Auto mode, which automatically regulates gas mixing based on culture demand. PID values: factory set default values.

Gas Control:

The gas control was set to 4-Gas mode, which automatically maintains DO and pH. The gas flow rate was based on the vessel size.

Up until day 3, gases were introduced into the vessel headspace only through the overlay port at a rate of 0.30 L/min using 4-Gas mixing to maintain pH and DO. On day 3, and for the remainder of the run, 5 - 10 ccm of gas were directly sparged into the system using a porous sparger and automatic 4 gas mixing. The overlay gas flow in the vessel headspace was kept at the previous settings.

A built-in sampling device enabled sterile sampling. Daily off-line measurements of glucose and lactate concentration were read using a YSI 2700, and cell density and cell viability was measured using an Automated Cell Counting System (NBS NucleoCounter™).

24.4 Results and Discussion

All data was logged via BioCommand® Batch Control PC-compatible Supervisory Control and Data Acquisition (SCADA) software (New Brunswick Scientific). As shown in Figure A, the CHO cells in this study grew steadily, reaching a maximum viable cell density of 5.55 x 10^6 cells/mL on day 5. Cell viability, shown in Figure B, ranged between 97.1 – 97.9% through Day 5, until the nutrient source, glucose, was depleted from the medium (as shown in Figure 3).

Figure C shows glucose consumption vs. lactate production during the process. As expected, lactate production steadily increased as the available glucose in the medium was consumed. As glucose in the medium become exhausted, consumption of lactate as a secondary carbon source also declined.[1]

This data demonstrates that the CelliGen BLU bioreactor is an easy-to-use, efficient system for the culture of CHO cells. No effort was made to optimize either the medium or the cell culture process control parameters. This study was only intended to document a general guide to bioreactor setup and operation, and present typical results you could expect to achieve with your mammalian cell line. For protocols on other cell lines, or for additional information on the CelliGen BLU, see www.nbsc.com/BLU.

Figure A: Cell growth over the 7-day run

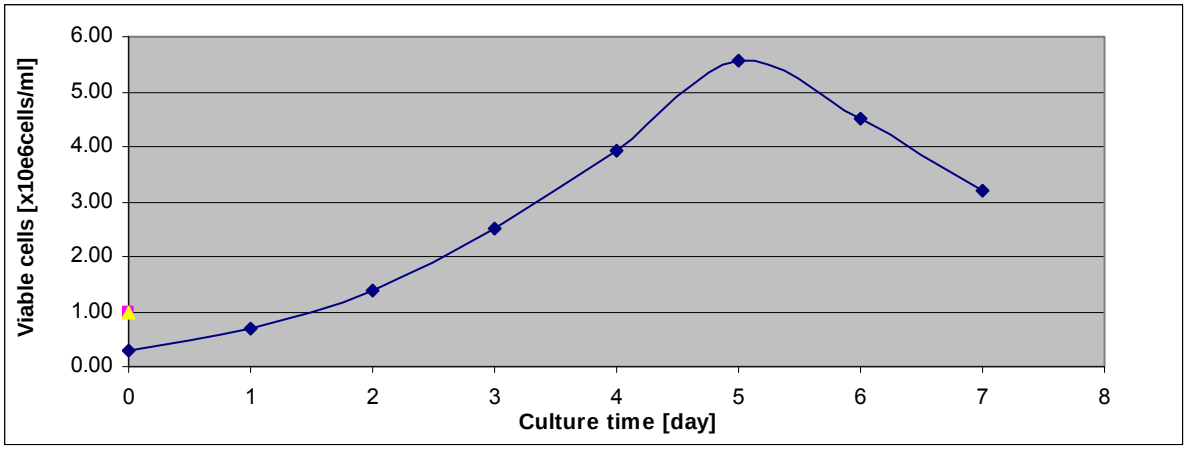


Figure B: Cell viability over the 7-day run

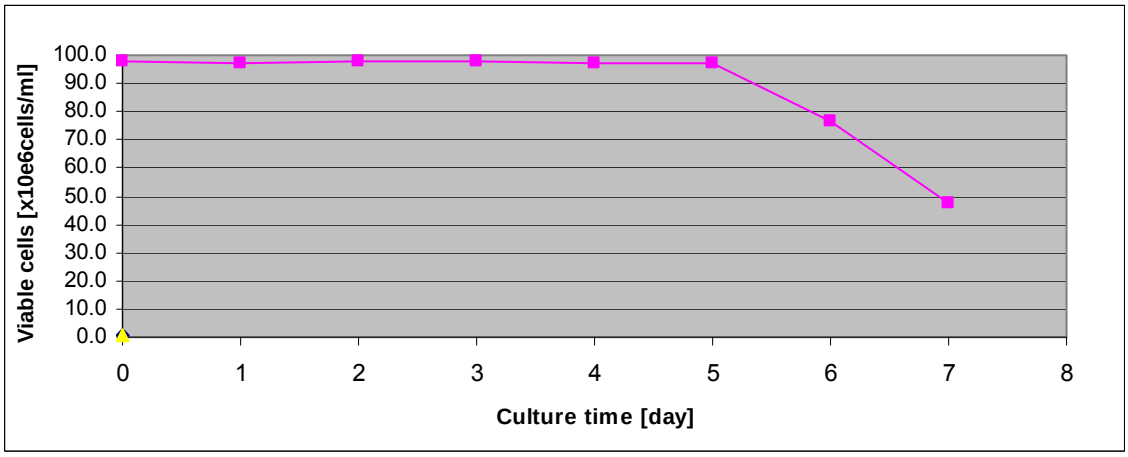
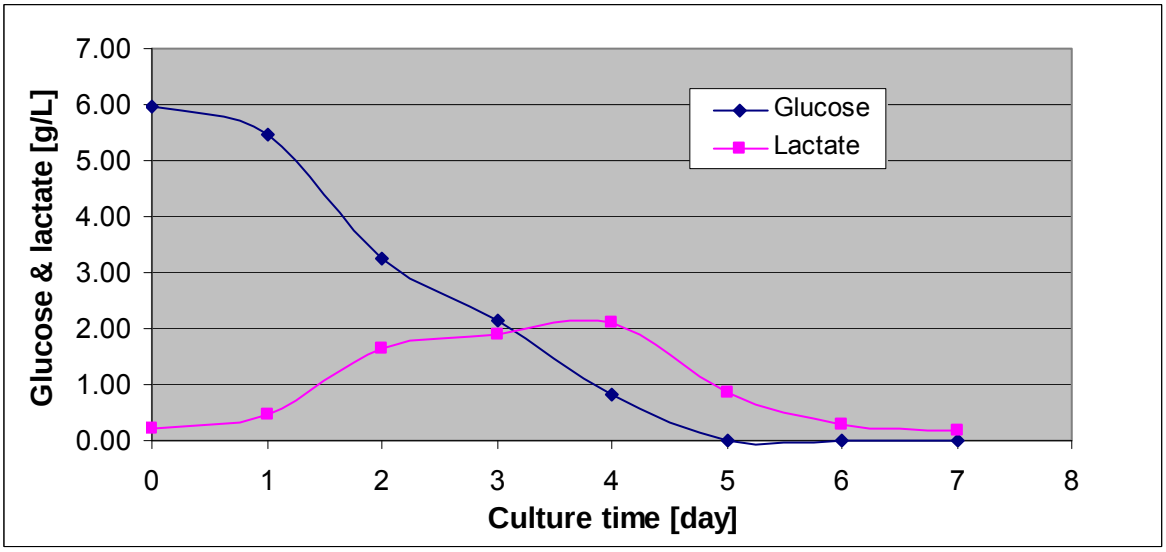


Figure C: Glucose consumption and lactate production over the 10-day run



1 Reference: "A single nutrient feed supports both chemically defined NS0 and CHO fed-batch processes: Improved productivity and lactate metabolism" by Ma, N., Ellet, J., Okediadi, C., Hermes, P., McCormick, E. & Casnocha, S. **Biotechnol Prog.**, 2009 Jul 27.

Authors of this article:

Guozheng Wang, Sr., Research Scientist, R&D Lab
Wenying Zhang, Research Scientist Assistant, R&D Lab
Rich Mirro, Product Manager
Vikram Gossain, Applications & Training Manager
New Brunswick Scientific

25 APPENDIX E: PRODUCT RETURNS

25.1 Return Procedure

As explained in Section 19.2, should you need to return your CelliGen BLU to NBS for any reason, first contact Customer Service to obtain a Returned Material Authorization (RMA) number. This number must appear on the outside of the shipping container, otherwise NBS Receiving will refuse to accept the shipment.

In addition, you must also certify that the instrument being returned has been thoroughly cleaned and decontaminated. A form for this purpose is provided on the following page; you can photocopy it and fill it out by hand. It can also be downloaded from our website (www.nbsc.com), if you prefer to fill it out electronically.

A copy of the completed Return Authorization and Decontamination Certificate must be attached to the outside of the container, with a second copy packed inside with the instrument.

25.2 Return Authorization and Decontamination Certificate

A sample form for you to copy and fill out is provided on the following page.

New Brunswick
an eppendorf company

Equipment Return Material Authorization (RMA) and Decontamination Certificate

Contact New Brunswick Scientific for an RMA number prior to returning any equipment, then complete this form and attach it to the outside container of the equipment being returned to our facility. In addition, attach a duplicate copy of the completed form to the item being returned.

Returned Material Authorization (RMA) Number _____

Equipment being returned: Model Number _____Serial Number_____

Reason for its return:

_____

_____

This equipment (check all that apply):

New Product
☐ Never used

Biohazards
☐ Not used
☐ Used, but decontaminated with
_____

Hazardous Chemicals
☐ Not used
☐ Used, but decontaminated with
_____

Radioactive Materials
☐ Not used
☐ Used, but decontaminated with
_____

I certify that the equipment described above has been thoroughly cleaned and decontaminated of all chemical, biological and radioactive contaminants and also certify that the returned unit is safe for unprotected human contact.

By: _____ _____
Signature Print name

Title: _____ Date: _____

Company: _____

Address: _____

_____

_____

Phone: _____ Fax: _____ Email: _____

Form 2847a

26 INDEX

4

4-Gas Mix, 75

A

Add User, 102
Adding Loops, 33
Addition Tubing
  Size of, 53
Aeration, 5
Agitation Speed Precautions, 104, 105
Agitation System, 4
Air Wash, 80
Air(1), 66
AirFlo, 75
Airflow Control
  Automatic, 5
  Manual, 5
Alarms
  ABS, 92
  Acknowledging, 94
  DEV, 92
Alarms History, 95
Alarms Screen, 40, 92
Analog Inputs & Outputs, 22

B

Bag Hanger, 7, 24
  Installing the, 24
BioCommand, 8, 22, 43, 106
Bioreactor Information Sheet, vii

C

Cabinet
  Cleaning of, 108
Calibration
  of Touchscreen, 28, 100
Calibration Screen, 37
Cascade
  Creating a, 61
Cascade
  Creating a, 62
Cascade Screen, 38, 62
CAUTION
  Symbol for, v
Cautions
  Recap of, 1
Certifications, 26, 27
CHO Cells
  Protocol for Growing, 139
Cleaning after a Run, 107
CO2(4), 66
CO2Flo, 75
Configuration Files, 98
Contact NBS
  How to, iii
Control Cabinet
  Installing the, 9
Control Cabinet Connections, 10
Control Loop
  Definition of, 134
Controller
  Definition of, 134
Copyright Notice, v
Corrosion Resistance, 138
CRUSH WARNING
  Symbol for, v

D

Decontamination Certificate, 145
Decontamination Certificate, 146
Deleting Loops, 35
Description of Vessel, 4
Disclaimer Notice, v
DO
  Control of, 6
DO Cascade, 63
DO Cascading, 57
DO Probe, 6
  Inspection of, 20
  Installation of, 21
Drawing Index, 129

E

Electrical Connections, 13, 15, 20, 21
Electrical Requirements, 13, 15, 20, 21
Exhaust Condenser, 6
Exhaust System, 6
Extended Warranty Option, vi

F

Feed Pumps
  To Add Liquid, 58
Fuse Replacement, 110

G

Gas Connections, 13
Gas Control
  0-1 TMFC, 65
  4 TMFCs, 75
Gas Overlay
  Operating Mode, 79
Gas Overlay Mixing, 79
Gauge Screen, 36
Growing CHO Cells, 139

H

Headplate Ports, 16
HOT SURFACE
  Symbol for, v

I

Index of Drawings, 129
Index of Tables, 131
Information Sheet, vii
Inoculation, 105
Inputs & Outputs, 22
Inspection
  of Boxes, 3
Installation
  Gas Connections, 13
International Offices
  List of, iii

L

Level Probes
  Application of, 58
Liquid Addition Systems, 52
Loading Pump Tubing, 51
Location
  Environment, 9
  Physical, 9
Loop Setpoints
  Entering the, 55
  Modifying the, 57

M

Main Screen Features, 29
Maintenance, 109
Maintenance Inspections, 109
Manual Conventions, v
Mass Flow Controller, 5
Media Temperature, 104
Modbus Com Port Pin Designation, 44
Motor Assembly
  Installation of, 21
Motor Replacement, 110

N

N2(3), 66
N2Flo, 75
NBS Offices, iii
NOTE
  Symbol for, v

O

O2(2), 66
O2Flo, 75
Operating Controls, 28
Overlay Gas Mixing Option, 7
OvlFlo, 79
OvlMix, 79

P

P&I Gains, 20
P&I Values
  Factory Settings, 55
  Setting, 55
pH
  Control of, 6
pH & DO Control via Gas Mixing, 65
pH Probe, 6
  Inspection of, 19

Installation of, 19
PID
Explanation of Constants, 135
Explanation of Tuning, 136
Plotting Trends, 81
Probe Calibration
Definition of, 135
Product Return Authorization, 112
Product Returns, 145
Protocol for Growing CHO Cells, 139
Pump Array
Standard, 86
Pump Assignment, 57
Pump Assignment Screen, 59
Pump Calibration, 58, 88
Pump Control Modes, 87
Pump Flow Rate, 88
Pump Period (sec), 89
Pump Setpoints, 86
Pumps Screen, 39

R

Read Line, 85
Recipe Files, 98
Regulatory Compliance, 26
Remove User, 102
Renaming Control Loops, 32
Return Authorization, 145, 146
Returned Material Authorization Number, 145
RMA, 145, 146
Rotameter, 5
RS232/422 Computer Interface, 43

S

Sampling System, 7
Saving a Process Configuration, 135, 136
Scales, 7
Security Keypad, 102
Serial Inputs & Outputs, 23
Service, 111
Service Connections, 10, 12
Service/Utility
Electrical, 13, 15, 20, 21
Setting Alarms, 92
Setup Screen, 40, 97
Shutdown after a Run, 107
Siliconizing the Vessel, 105
Specifications, 25
Stacking Pumps, 132
Start-Up Screen, 29
Supervisory Software, 8
Synoptic Screen, 31

T

Table Index, 131
Table of Contents, xii
Temperature
Control, 5
RTD, 5
Setpoint, 5
Temperature Control, 104
Touchscreen
Calibrating the, 28, 100
Installing the, 10
Trend Graph, 82
Creating a, 81
Trend Graph Buttons, 83
Trend Screen, 38
Trend Setup Screen, 82
Troubleshooting, 111

U

User Button, 102, 103
Utilities, 11

V

Validation Documentation, 26
Vessel
Description of, 4
Installation of, 21
Siliconizing the, 105
Vessel Pressurization, 14

W

WARNING
Symbol for, v
Warnings
Recap of, 1
Warranty, vi
Option for Extended, vi

Warranty Registration, vi

Z

Zoom Coordinates, 84